The Behavioural and Physiological Effects

of Pile-driving Noise on Marine Species

Submitted by William Thomas Corbett to the University of Exeter as a thesis for the degree of Masters by Research in Biological Sciences, September 2018.

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Abstract

Anthropogenic noise is a recognised pollutant in both terrestrial and aquatic environments. Noise levels in the ocean have risen greatly over the past few decades. The principal low-frequency background noise in the oceans is generated by commercial shipping, and can have a profound impact on fitness in a variety of marine species. Additionally, the loud impulsive noise from industrial pile-driving activity is increasingly prevalent in the oceans due to the recent development of offshore windfarms to meet renewable energy targets. Little research has explored the impact that this noise is having on marine species, especially invertebrates. The aim of this thesis is to explore the physiological and behavioural responses of marine species to playback of pile-driving noise, using playback of ambient marine sound as a control. The first study aimed to assess the physiological and behavioural response of the decapod crustacean *Carcinus maenas* to pile-driving noise playback in experimental tanks. In the physiological experiment, crabs did not significantly differ in their oxygen consumption or haemolymph parameters in response to pile-driving noise and ambient sound. However, in the behavioural feeding experiment, crab behaviour was significantly altered during pile-driving playback, including increased time spent immobile and decreased likelihood to feed. The second study aimed to assess the avoidance behavioural response of marine fish in their natural environment to pile-driving noise playback by use of a baited remote underwater video (BRUV) system coupled with a loudspeaker. Playback of pile-driving noise had a significant effect on the number of pelagic fish species surrounding the BRUV, indicating an avoidance effect. However, playback did not have a significant effect on the number of benthic fish species or species richness. Both these studies demonstrate that pile-driving noise causes behavioural changes in marine species, which could have potential fitness costs. Thus, exploration into ways of mitigating noise impacts when undertaking pile-driving activities in the ocean should be further explored.

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Table of Contents

List of Figures and Tables

Chapter 1

A Review of Anthropogenic Noise in the Marine Environment

1.1 Introduction

The global human population is estimated to be over 7 billion and is still increasing. It is expected that within the next century the population will reach 11 billion people worldwide (UN, 2015). The current population size is already a cause for concern due to the pressure it exerts on the environment, which is crucial for food production and water, as well as the air we breathe.

At present, humans are having a large impact on the environment; some scientists have even termed the impact we are having on the environment as a new "epoch" – the Anthropocene (Zalasiewicz *et al.*, 2008). Throughout recent history, humans have had a very noticeable effect on the terrestrial environment, resulting in species becoming extinct through drivers such as habitat loss, exploitation and chemical pollutants (Millennium Ecosystem Assessment, 2005). One of the more concerning impacts is that of human driven climate change due to an increase in greenhouse gases. This is causing widespread changes in the climate, increasing the prevalence of extreme climatic events and resulting in higher surface temperatures of the earth. On average surface temperatures have warmed by 0.85 °C from 1880 to 2012 (IPCC, 2013). Humans are directly impacted by climate change due to the resulting reduction in crop yields, affecting some of the poorest regions of the world most severely (Research Institute (IFPRI), 2009).

It is not just the terrestrial environment that climate change is negatively affecting. In the marine environment, summer Arctic ice has reduced consecutively each decade due to rising sea temperatures and it is showing no signs of slowing (Wadhams, 2012). Increases in sea temperatures in combination with ocean acidification (a result of CO_2 emissions), are also degrading the most biodiverse parts of the ocean: tropical coral reefs (Hoegh-Guldberg *et al.*, 2007). Mass coral bleaching is becoming increasingly frequent on coral reefs, with an increasing likelihood of annual bleaching events on many reefs in the near future (Hughes *et al.*, 2018), threatening their long-term survival.

The threats to the ocean are not limited to just warming seas and ocean acidification. Other threats include overfishing, invasive species and plastic. With the development of high-tech fishing fleets, fish are often depleted faster than they can be replenished, exemplified by the Atlantic cod (*Gadus morhua)* collapse in 1992 (Hutchings and Myers, 1994). Unfortunately, overexploitation of fish stocks can be seen in every ocean. Estimates suggest that large predatory fish biomass is at a mere 10% of pre-industrial levels (Myers and Worm, 2003). Invasive species such as the Indo-Pacific lionfish species (*Pterois spp.*) have dramatically changed Atlantic coral reef fish populations. In 2010, lionfish were thought to have comprised nearly 40% of the total predator biomass (Green *et al.*, 2012), putting pressure on an already fragile ecosystem. Waste plastic in the oceans is another significant threat; from production on land, plastics have been found in Arctic sea ice (Barnes *et al.*, 2009) and deep-sea sediments (Van Cauwenberghe *et al.*, 2013). Both macro and micro (< 5 mm) plastics can be ingested by fish, sea birds, turtles, marine mammals and even invertebrates (Lusher *et al*., 2013). Ingestion of these can cause physical blockages or strangulation. Plastics are also efficient vectors of dangerous organic contaminants such as polychlorinated biphenyls (PCBs) which adhere to the plastic in both the production stage and in the environment (Teuten *et al.*, 2009).

In the terrestrial environment a more novel threat, human generated noise, termed anthropogenic noise, is now recognised as a pollutant. This is in part due to the increased prevalence of noise exposure in the last century due to growth in transportation networks, resource extraction, motorised recreation and urban development (Barber *et al.,* 2010). Noise pollution is having a negative effect on a variety of different terrestrial species including birds, amphibians and even humans (Fritschi *et al.*, 2011; Shannon *et al.*, 2015). However, anthropogenic noise does not just threaten the terrestrial environment, it is also recognised as a significant threat to the world's oceans (Tasker *et al.*, 2010; Gedamke *et al.*, 2016).

1.2 Ambient Sound in the Ocean

French oceanographer Jacques-Yves Cousteau titled his famous book and later Academy Award winning film "*Le Monde Du Silence*", which translates as "*The Silent World*". This was one of the first films to use underwater videography to

show the oceans' vibrant colours and life beneath the waves. However, the underwater world is not as silent as the film title suggests. Human ears are adapted for use in air, not water, which can give the perception of the ocean being a "*Silent World*". In fact, use of specialist sound-detection equipment reveals a cacophony of noises underwater.

Sound waves are produced by movement or vibration of an object in a medium such as air or water (Hawkins and Popper, 2016). Water is a very efficient medium for sound to travel in due to its high molecular density. Sound travels approximately five times faster in water than in air, and attenuates far less over the same distance, allowing underwater sound to propagate much further from the source (Slabbekoorn *et al.*, 2010). Sound underwater has two components: sound pressure and particle motion. When a sound wave travels through water, the hydrostatic pressure fluctuates as the water is compressed and decompressed (Carroll *et al.*, 2017). This is sound pressure and can be detected by using a hydrophone. Underwater acoustic pressure is usually reported in decibels (dB) with respect to a reference sound pressure value of 1 µPa (Popper *et al.*, 2014). Particle motion is the oscillatory motion of particles back and forth from a vibrating source, allowing propagation of vibratory energy, and is recorded using an accelerometer (Nedelec *et al.*, 2016). Particle motion can also indicate the direction of the propagating sound wave, and is often expressed in three ways: displacement (m), velocity (ms^{-1}) or acceleration (ms^{-2}) (Nedelec *et al.*, 2016).

Ambient underwater sound comes from a variety of sources. Abiotic sound is the sound generated by physical processes such as the wind, the waves it produces, ice movement, precipitation, thunder, lightning and even the occasional earthquake (Hildebrand, 2005). Wind has the broadest of frequency ranges between 1 Hz and 100 kHz; below 10 Hz, surface waves dominate the ambient sound spectrum. Biotic sound sources, which come from a vast number of different species, also contribute to the ambient sound of the ocean.

Mammalian whale song is major source of biotic sound in the open ocean, and the mysticetes sub-order (baleen whales), typically produce calls at low frequencies. For instance, the blue whale (*Balaenoptera musculus*) typically calls at frequencies between 8 and 25 Hz (Stafford *et al.*, 1998). Blue whales produce these calls in a similar way to humans by passing air over vocal chords in the

larynx (Adam *et al.*, 2013). The other sub-order of cetaceans are the odontocetes (toothed whales), which includes dolphins, porpoises and the sperm whale (*Physeter macrocephalus*). Typically odontocetes produce sound at mid and high-frequencies between 1 and 200 kHz (Matthews *et al.*, 1999). Like the mysticetes, odontocetes can call using their larynx, but a more widely recognised sound is their high-frequency echolocating sonar call. This call is produced by passing air through a structure in the head called phonic lips (Cranford *et al.*, 2011). It is this that allows bottlenose dolphins (*Tursiops spp.*) to emit short clicks with peak frequencies as high as 120 to 130 kHz (Au, 2004). Mammalian pinnipeds (seals) are also known to produce many sounds underwater; depending on the species, the frequencies observed range between 0.02 and 20 kHz (Wartzok and Ketten, 1999). Clicks have been known to be produced but are not believed to be a form of echolocation (Schusterman *et al.*, 2000).

It is not just cetaceans and pinnipeds that produce biotic sound in the ocean, as fish are able to also (Ladich, 2014). Gadoid fish, such as Atlantic cod can produce low-frequency grunts by drumming their swim bladder with the muscles attached to it (Hawkins and Rasmussen, 1978). Indirect vibration of the swim bladder can be seen in other fish, such as the Atlantic croaker (*Micropogonias undulatus*), by use of tendons or bony plates (Ladich and Fine, 2012). Other species, such as clownfish (*Amphiprion spp.*) can produce sound by use of pharyngeal structures, generating frequencies between 450 and 850 Hz (Parmentier *et al.*, 2007).

Some marine invertebrates are also capable of producing sounds. Snapping shrimp (*Alpheus spp.* & *Synalpheus spp.*) are often the dominant source of mid-frequency ambient sound in the ocean (Hildebrand, 2009), especially on reefs. They are able to produce this sound by rapidly closing their front claws causing a cavitation bubble which generates a click with a peak frequency between 2 and 5 kHz (Au and Banks, 1998). Lobsters (*Panulirus argus* and *Homarus americanus*) are also able to produce sound by stridulating their antennae (Bouwma and Herrnkind, 2009; Ward *et al.*, 2011).

1.3 Sound Reception and Use in the Ocean

The oceans are vast, and so present challenges in navigation and communication with conspecifics. It is often turbid, and/or often dark, with very little significant light below 200 m and none below 1,000 m (NOAA, 2017), and the transport of

olfactory cues depends on currents. Being able to hear (detect sound waves) underwater is key for many marine animals, allowing them to “view”, or interpret, the underwater world around them more easily. Marine animals can hear in a surprising variety of ways and for a number reasons.

Both sub-orders of whales, the odontocetes and mysticetes, have hearing adapted to underwater life. Odontocetes are understood to be able receive sound to their inner ears by channelling soundwaves through their lower jaw bone and the specialised fats associated with it (Koopman *et al.*, 2006). Much of the research into hearing capabilities for odontocetes is based on captive delphinids; most of which are thought to have hearing between 0.02 and 100 kHz, with some species being able to hear as high as 200 kHz (Hildebrand, 2005). Less is known about the hearing capabilities of mysticetes whales, but recent research into fin whales (*Balaenoptera physalus*) suggests that they can hear low-frequency sound by transferring vibrations through their skull to their inner ears (Cranford and Krysl, 2015). Mysticete hearing capabilities, based on modelling, are understood to range from between 0.02 and 30 kHz, with some species hearing sounds low as 10 Hz (Hildebrand, 2005). Beyond cetacea, other marine mammals such as pinnipeds (seals) and sirenia (dugongs and manatees) are also capable of hearing underwater to different degrees (Wartzok and Ketten, 1999).

Sound is crucial to the successful existence of marine mammals, as it allows them to sense their surroundings. A well-known example is echolocation in odontocetes. Ultrasonic clicks are emitted into their environment and are reflected back off objects (echoes), allowing the whales to determine distances to an object of interest (Wartzok and Ketten, 1999). This is useful when navigating in low visibility environments and predating upon fish. Clicks, pulses and a variety of other sounds are associated with different behaviours between odontocetes. For example, bottlenose dolphins (*Tursiops truncatus*) use sound for a number of social interactions such as high-frequency burst–pulse sounds during aggressive interactions with conspecifics (Blomqvist and Amundin, 2004). Mysticetes whales are also known to have different calls at lower frequencies than the odontocetes; these calls are often associated with different behaviours such as feeding, courtship and parental care (Edds-Walton, 1997; Cerchio and

Dahlheim, 2012). Only male fin whales sing, indicating a likely function of attracting females, potentially from a great distance (Croll *et al.*, 2002).

Marine fish are also able to detect sound underwater. There are two sensory systems for detection of sound in fish: the inner ear and the lateral line system (Slabbekoorn *et al.*, 2010). Fish can detect the particle motion element of sound due to specialised structures made of calcium carbonate called otoliths (ear bones) in the inner ear. The otoliths are much denser than, and therefore move slower than, both the rest of the fish and the surrounding water in response to sound. This slower movement of the otoliths displaces the cilia on the hair cells in the inner ear and is detected as sound by the brain (Ladich, 2014). Whilst most marine teleost fish are likely to detect the particle motion element of sound, some are also able to detect sound pressure. Many marine teleosts also have a gas-filled swim bladder, the main function of which is to provide buoyancy in the water column. The gas filled swim bladder is less dense than the surrounding water and so is easily compressed by sound waves. These oscillations of the swim bladder are transmitted to the inner ear and are detected as sound (Ladich, 2014). Fish can also detect sound waves by use of the lateral line system. The lateral line is situated on either side of the fish and consists of sensory cells called neuromasts. The neuromasts can also detect particle motion like the inner ear (Ladich, 2014). Some fish can detect a wide range of frequencies of sound, but the majority of fish can detect frequencies between 50 and 1500 Hz (Popper and Hastings, 2009).

Like many marine mammals, fish also utilise sound underwater to their advantage. Sound is often used by fish to communicate with conspecifics, such as during courtship. Haddock (*Melanogrammus aeglefinus*), for instance, produce a range of sounds including a variety of “knocks” during courtship interactions (Hawkins *et al.*, 2000). The Lusitanian toadfish (*Halobatrachus didactylus*) attracts mates to their benthic nests with a tonal advertisement call (Amorim *et al.*, 2011). Fish use sound to communicate not only for courtship purposes. For example, the Hawaiian domino damselfish (*Dascyllus albisella*) produces a range of pops, chips and pulses towards conspecifics in aggression (Mann and Lobel, 1998). Sound has also been shown to be an important cue for settlement-stage coral reef fish in finding suitable habitat. Studies have demonstrated that larval fish preferred to settle on patch reefs and are attracted

to light traps with playback of reef sound compared to no sound playback (Simpson *et al.*, 2004, 2005). The settlement-stage coral reef fish also preferred high-frequency sound (indicative of marine invertebrates) than low-frequency sound (predominantly fish vocalisations) on patch reefs (Simpson *et al.*, 2005), suggesting that invertebrate sound could indicate a more suitable habitat in which to settle.

Some marine invertebrates can also detect the particle motion element of sound waves underwater. Particle motion detection by invertebrates may involve mechanoreceptors of three types: superficial receptor systems, statocyst receptor systems and chordotonal organs (Budelmann, 1992). Superficial receptor systems are the sensory hairs found all over the external body surface of invertebrates (Budelmann, 1992). Statocyst receptor systems, which work similarly to the otoliths of the inner ear of fish are primarily used as accelerometers for gravity detection, but may also be able to detect particle motion in invertebrates (Roberts and Elliott, 2017). Chordotonal organs in the joints of crustacea also appear to be sensitive to vibrations (Roberts and Elliott, 2017). The common prawn (*Palaemon serratus*), for example, has been shown to be responsive to frequencies ranging from 100 to 3,000 Hz (Lovell *et al.*, 2005).

Sound underwater is also thought to play an important role for invertebrates, especially in avoiding predators. Both American lobsters (*Homarus americanus*) and Caribbean spiny lobsters (*Panulirus argus*) stridulate their carapaces in the presence of predators as a defence mechanism to deter them (Bouwma and Herrnkind, 2009; Ward *et al.*, 2011). Reef sound is thought to play a key role in habitat selection in coral larvae, as they have been shown to initiate settlement behaviour in choice chambers when exposed to playback of coral reef sound (Vermeij *et al.*, 2010). Holoplanktonic crustaceans appear to prefer to avoid reef sound, possibly to avoid predators on the reef, while settlement-stage meroplanktonic crustaceans responds positively to reef sound (Stanley *et al.,* 2010; Simpson *et al.*, 2011). Sound has been hypothesised to be important for crustaceans when finding food. The pandalid shrimp (*Pandalus borealis*) inhabits the deep benthic regions of the Atlantic and Pacific Oceans where light is limited, and these deep-sea scavengers are thought to be able to detect sound from a carcass hitting the sea bed hundreds of meters away in order to feed (Klages *et al.*, 2002). Cleaner shrimp (*Ancylomenes longicarpus*) are also thought to utilise

sound by clapping to attract clients, shrimp that clap more frequently are more likely to attract and clean a client (Chapuis and Bshary, 2010).

A great diversity of marine taxa rely on sound to attract mates, find food or avoid being eaten themselves. Therefore, anthropogenic noise in the marine environment is likely to impact this crucial sensory modality for marine fauna and could have an impact on the long-term survival of many taxa.

1.4 Anthropogenic Noise in the Ocean

Noise is of growing concern in the marine environment due to increased human activity generating a range of different sounds. The effects of anthropogenic noise are beginning to show a negative impact on many marine species; for example, the use of sonar has been linked to mass stranding of whales (Amico *et al.*, 2009; Goldbogen *et al.*, 2013). However, anthropogenic noise in the oceans is not exclusively from sonar, but is generated from a wide range of human related activities which can be categorised as direct and indirect sources. Direct sources of noise are deliberately introduced into the oceans, including sonar for exploration and fish-finding, seismic surveys for geo-prospecting and deterrents on fishing gear and at industrial sites. Indirect sources are by-products of human activities in the oceans, which include shipping, motorboats, military activity and industrial activity (pile-driving and drilling). Noise can also be divided into continuous and impulsive noises; continuous noise includes ship noise and operational noise of offshore renewable energy devices, while impulsive noises, such as seismic airguns, don't last for extended periods of time, but can often be extremely loud. Each of these different anthropogenic noises when added to the ambient ocean soundscape can have a real impact in a variety of ways on a wide range of taxa.

Commercial shipping involves ~50,000 large vessels that transport over 90% of world trade around the world's oceans, and the noise generated by ships is now the principal source of low-frequency (5–500 Hz) background noise in the oceans (Hildebrand, 2005). Over the past few decades, ship noise has increased the background noise levels of the ocean by as much as 12 dB re 1 μPa RMS, in part due to the increased number and size of the shipping fleet (Hildebrand, 2009). The noise generated by large vessels is predominantly from cavitation bubbles at the tips of the propellers, as a result of a static pressure drop below ambient

water pressure (Hildebrand, 2009). However, noise is also generated by both the machinery onboard and hydraulic flow over the hull (Hildebrand, 2005).

With increased prevalence of large vessels in the ocean, a growing number of studies have explored the impact of shipping noise on marine life (Slabbekoorn *et al.*, 2010). Ship noise is generally low-frequency, as is the frequency of the communication calls of mysticete whales, and so it is thought to be causing a masking effect of whale calls. It has been suggested that some mysticete species change their call frequencies and duration in response to shipping noise (Parks *et al.,* 2007; Castellote *et al.,* 2012). Both masking and changes in call frequency could reduce the range and effectiveness of their communication with conspecifics (Tyack, 2008), possibly resulting in reduced breeding success for populations. Masking by ship noise could also be a problem for fish that communicate acoustically (Radford *et al*., 2014). For example, the Lusitanian toadfish (*Halobatrachus didactylus*) uses calls to attract a mate, but ship noise frequencies overlap with the most sensitive hearing range frequencies of the toadfish (Vasconcelos *et al*., 2007). Ship noise does not only have a negative effect through masking of marine life; it can impact behaviour also (Slabbekoorn *et al.*, 2010). In tank-based experiments, fish display compromised antipredator behaviour in response to ship noise. European eels (*Anguilla anguilla*) were shown to be less likely and slower to startle to a simulated predator when exposed to ship-noise playback, and were caught twice as fast with a simulated pursuit predator (Simpson *et al*., 2015). Bluefin tuna (*Thunnus thynnus*) in fixed tuna cages demonstrated uncoordinated swimming behaviour in response to a car ferry passing (Sarà *et al.*, 2007), which could leave them more vulnerable to predation in the open ocean. Feeding behaviour was also shown to be disrupted in three-spined sticklebacks (*Gasterosteus aculeatus*) in tank-based experiments, where exposure to ship-noise playback resulted in significantly fewer live *Daphnia magna* being consumed as a consequence of more handling errors (Voellmy *et al.*, 2014). Crustaceans have also been shown to become behaviourally compromised in tank-based experiments with ship noise playback. For example, the common shore crab (*Carcinus maenas*) was slower to retreat to shelter with a simulated predator attack and feeding was also more likely to be disrupted with ship noise playback (Wale *et al.,* 2013a).

Impacts of ship noise are not limited to changes in behaviour in marine taxa. There are potential sublethal physiological effects which could carry fitness consequences for populations. Physiological effects in response to ship noise include an elevated ventilation rate in crabs and fish (Wale *et al.*, 2013b; Simpson *et al.,* 2015; Purser *et al.*, 2016), which indicates stress and can be linked to an increase in metabolic rate. Another sublethal effect that has been detected experimentally is an increase in the glucocorticoid cortisol in the blood of freshwater fish when exposed to ship noise (Wysocki *et al.,* 2006). Cortisol is a stress hormone which if secreted for a prolonged period can have negative effects on fish health (Pottinger, 2008). This is also thought to be true in North Atlantic right whales (*Eubalaena glacialis*) exposed to ship noise in the Bay of Fundy (Canada), where in the aftermath of the 9/11 terrorist attack in 2001 there was a 6 dB reduction in sound in the Bay due to reduced ship traffic (Rolland *et al.*, 2012). At the same time there was also a decrease in baseline levels of the stress related glucocorticoid hormones in whale faecal matter, implying ship noise may cause a chronic stress response in the whales.

It is not only large vessels that are of a concern in the ocean, other smaller seafaring craft are also having an impact on the marine environment. Motorboat noise is very prevalent around coastal regions where smaller craft are used for both commercial and recreational activities, using both inboard and outboard engines. Motorboats produce noise in a similar way to shipping noise, with much of the noise coming from cavitation bubbles on the propellers. This noise is generally most intense in the mid-frequency range (1–5 kHz; Hildebrand, 2009).

Motorboat noise has been demonstrated to have a variety of deleterious effects on coral reef fish. It can directly affect the anti-predator behaviour of fish, compromising survival. In one experiment, Ambon damselfish (*Pomacentrus amboinensis*) were 2–3 times more likely to be predated on by a fish predator during motorboat-noise playback in a tank and when exposed to real motorboats in open water (Simpson *et al.,* 2016a). Feeding behaviour can also be altered, with lower feeding frequencies associated with greater motorboat traffic in Mediterranean damselfish (*Chromis chromis;* Bracciali *et al.*, 2012). Parental care behaviour of the spiny chromis (*Acanthochromis* polyacanthus) was altered *in situ* when exposed to motorboat noise playback, with serious fitness consequences whereby some of the exposed nests experienced complete brood

mortality (Nedelec *et al.*, 2017). Settlement-stage fish recruitment to reefs is also affected. Research showed a masking effect of ambient reef playback by playback of motorboat noise in choice chamber experiments with *Apogon doryssa* (Holles *et al.*, 2013), and a reduction in settlement of fish to patch reefs when motorboat noise is added to ambient reef sound recordings (Simpson *et al.,* 2016b). Other behaviours have been observed to change in reef fish exposed to motorboat noise, including reduced boldness, movement and time spent caring for their nests (Picciulin *et al.*, 2010; Holmes *et al.*, 2017). Other than reef fish, behavioural changes have also been seen in bluefin tuna when exposed to motorboat noise; in fixed traps, bluefin tuna showed more agonistic behaviour to conspecifics when motorboats passed (Sarà *et al.*, 2007).

The majority of research to date on the impacts of motorboat noise has explored behavioural impacts, but, there are other concerning sublethal responses of fish including altered hearing capabilities (Scholik and Yan, 2002), increased cardiac output (Graham and Cooke, 2008) and elevated metabolic rate (Simpson *et al.,* 2015, 2016a); the latter two are indicative of a stress response. However, it is not just fish that are thought to be impacted by motorboat noise; there is some evidence that marine invertebrates are also affected. Early life stage sea hares (*Stylocheilus striatus*) exposed to motorboat noise *in situ* were less likely to develop successfully and had increased mortality (Nedelec *et al.*, 2015). If true for natural populations, motorboat noise could have direct fitness consequences for sea hares and possibly other marine invertebrates.

Air guns used in seismic surveys are a particularly intense and impulsive form of noise pollution and have been researched for potential impacts on marine life. Seismic surveys are used to explore for oil in the oceans, mapping the earth's crust below the seabed. Such surveys employ an array of air guns (normally 12–48) which simultaneously release large volumes of pressurised air, creating loud explosions (technically implosions) which reflect off the seabed and deeper oil and gas reserves, and are detected by towed hydrophones on the surface (Hildebrand, 2009). The impulsive noise produced is at a low frequency (majority between 10–300 Hz) and a very high intensity (Carroll *et al.*, 2017).

Worryingly, the loud impulsive noise of seismic surveys is also thought to pose a significant threat to marine life. Behaviourally it has been shown to cause animals to avoid areas close the noise; a 78% decline in reef fish was observed on a

North Carolina reef after seismic surveying with air guns nearby (Paxton *et al.*, 2017). Humpback whales (*Megaptera novaeangliae*) were also found to avoid air gun arrays within 3 km at source levels over 140 dB re 1 µPa (Dunlop *et al.*, 2017) and male fin whales were recorded to leave the Alboran basin near the Straights of Gibraltar within 72 hours of a 10-day seismic survey and not return until a few days afterwards, as well as adapting their calls during the disturbance (Castellote *et al.,* 2012). Physiological changes due to seismic airgun noise have also been observed in fish. In one study, two freshwater fish species were shown to have auditory hearing threshold shifts after exposure to air-gun noise (Popper *et al.*, 2005), and playback of airgun noise in experimental tanks resulted in elevated ventilation rates and longer-term habituation to noise in European sea bass (*Dicentrarchus labrax;* Radford *et al.,* 2016). Invertebrates are also affected, with developmental delays and body abnormalities seen in scallops (*Pecten novaezelandiae*) when exposed to playback of airgun noise (De Soto *et al.*, 2013). More worrying is the link between seismic surveying and mortalities of marine life, as it has been linked to be the cause of mass stranding events of the giant squid (*Architeuthis dux*), with individuals displaying extensive tissue damage (Guerra *et al.*, 2011). However, not only large invertebrates are at risk, as zooplankton experimentally exposed to air guns *in situ* suffered a two to three-fold increase in mortality of adult and larval zooplankton in net tows, with total mortality of all larval krill (McCauley *et al.*, 2017). This has serious implications for ecosystem functioning as almost all marine animals rely directly on zooplankton as a source of food, or on food webs underpinned by zooplankton.

The different impacts of anthropogenic noises outlined above, while not exhaustive, demonstrates a spectrum of detrimental effects on a wide range of marine fauna. As the human population continues to increase, anthropogenic noise is only likely to become more prevalent in the ocean from a growing number of different sources. At present, there is a global shift away from a reliance on fossil fuels, and a growing adoption of renewable technologies, including offshore installations, to produce energy. Construction and operation of these installations will have a further impact on the ambient ocean soundscape. Better understanding of how this may affect marine life is key for developing mitigation strategies to lessen the impact they could have.

1.5 Research Aims

In the field of anthropogenic noise research, much of it has explored the effect of both commercial shipping noise and motorboat noise. A relatively small amount of research has looked at the impact of pile-driving in the oceans on marine fish (Mueller-Blenkle *et al.*, 2010; Radford *et al.*, 2016) and marine mammals (Brandt *et al.*, 2011), and even less so on the impact of pile-driving noise on marine invertebrates (Roberts *et al.*, 2016). The aim of this Masters research was to further the understanding of pile-driving on both the physiological and behavioural responses of marine species.

The first study (Chapter 3) conducted in tanks in the laboratory was split into two parts and explored the physiological and behavioural responses of a marine invertebrate, the common shore crab, to the playback of pile-driving noise. Crabs were used in the laboratory to avoid unnecessary use of vertebrates and the need for Home Office licencing. Shore crabs have also been used effectively in earlier tank-based studies exploring effects of anthropogenic noise (Wale *et al,.* 2013a, 2013b). In the physiological study, oxygen consumption was measured, and haemolymph was taken for analysis of glucose concentration, protein concentration and pH. It was hypothesised that crabs in the pile-driving treatment, in comparison to the ambient treatment, would have increased oxygen consumption rates and differing haemolymph parameters. These measures would be expected to change due to noise in general already being a recognised stressor in the environment and these physiological measures being indicators of stress. In the behavioural study, feeding behaviour was compared when exposed to both pile-driving noise and ambient sound playback. A variety of different measures were taken including time spent immobile, whether they emerged and whether crabs made foraging mistakes. It was hypothesised that the behaviour of the crabs may be significantly altered due to distraction or stress in response to the pile-driving treatment.

The second study (Chapter 4) was conducted in the field, looking at the behavioural effect of playback of pile-driving noise in a more natural environment on wild populations of marine fish. This study was carried out on wild populations of fish, taking advantage of the local diversity of fish at the field sites, with a range of observable behaviours, but avoiding Home Office issues since the fish were not studied in captivity. A BRUV (baited remote underwater video) system in

conjunction with sound playback was used to determine the marine community-level response. Numbers of fish for benthic and pelagic species and species richness were measured pre- and post-playback track change (continued ambient playback or a change to pile-driving playback). It was hypothesised that there would be a significant decrease in community composition when exposed to the pile-driving treatment. This is expected as pile-driving noise playback would deter some species from surrounding the BRUV system.

Chapter 2

The Physiological and Behavioural Response of *Carcinus maenas* to Pile-driving Noise

2.1 Introduction

Anthropogenic noise is of growing concern in the marine environment. The major source of anthropogenic noise in the oceans comes from shipping vessels, with the noise generated by ships now the principal source of low-frequency background sound (Hildebrand, 2005). Much effort has gone into researching the impact this prevalent noise is having on marine life (Kunc *et al.*, 2016). However, a growing noise source in the ocean, with fundamentally different characteristics is pile-driving noise generated from construction of offshore wind farms, ports and bridges (Popper and Hastings, 2009). The most common technique used is impact pile-driving. This technique consists of a large weight repeatedly being dropped against a pile (steel or reinforced concrete pole), thus driving it into the seabed. This results in a loud impulsive noise in both the water column and seabed which can reach sound pressure levels greater than 250 dB re 1µPa at the source (Gotz *et al.*, 2009), with the majority at lower frequencies between 100 and 1,000 Hz (Gotz *et al.*, 2009; Hildebrand, 2009). Pile-driving activity is most likely to increase as countries increase offshore wind farm development in order to reach renewable energy targets, causing great concern for the health of marine ecosystems.

Current research into the effects of pile-driving on marine life is varied, but predominantly focuses on fish. Behavioural effects of pile-driving activity on Dover sole (*Solea solea*) and Atlantic cod (*Gadus morhua*) have been observed in large net pens, with an increase in both swimming speeds and freezing motion for each fish respectively compared to no playback (Mueller-Blenkle *et al.*, 2010). In a laboratory study with juvenile European seabass (*Dicentrarchus labrax*), fish were less cohesive and directionally ordered as a group in response to pile-driving playback (Herbert-Read *et al.*, 2017). Avoidance behaviour in response to pile-driving activity and playback has also been observed, with schools of European sprats (*Sprattus sprattus*) more likely to disperse, and those of Atlantic mackerel (*Scomber scombrus*) more likely to change depth (Hawkins *et al.*, 2014). Avoidance has also been observed in grey snapper (*Lutjanus griseus*) in

response to the onset of nearby pile-driving activity (Iafrate *et al.*, 2016). In response to pile-driving noise playback in the laboratory, physiological effects have also been observed, including increased ventilation rates (Poulton *et al*., 2016; Radford *et al.*, 2016; Spiga *et al*., 2017) and reduced oxygen consumption (Debusschere *et al.*, 2016), both indicators of a stress response. Six different freshwater fish (*Oncorhynchus tshawytscha, Acipenser fulvescens, Oreochromis niloticus, Oreochromis mossambicus, Trinectes maculatus* and *Morone chrysops x Morone saxatilis)* exposed to simulated pile-driving showed physical damage to the swim bladder, hair cells in the inner ear and other injuries including haematomas and herniations (Halvorsen *et al.*, 2012a, 2012b; Casper *et al.*, 2013a, 2013b, 2017). More worryingly, in some cases, simulated pile-driving resulted in mortalities (Halvorsen *et al.*, 2012b; Casper *et al.*, 2013). However, this may not be realistic as in the ocean fish can freely move away from the source of activity. Not only have fish been influenced behaviourally by pile-driving activity in the ocean; a study on harbour porpoises (*Phocoena phocoena*) showed that acoustic activity ceased entirely one hour after pile-driving activity and stayed below normal levels of activity for three days up to 2.6 km away from the initial piling activity (Brandt *et al.*, 2011).

While there is growing evidence of the impacts of pile-driving noise on fish and marine mammals in the marine environment, there is only a very limited amount of research into the possible impacts of pile-driving noise on marine invertebrates. One study looked at the effect of pile-driving on the behaviour of the blue mussel (*Mytilus edulis*) and the common hermit crab (*Pagurus bernhardus*). In these experiments, blue mussels exhibited significantly different physiology and behaviour in comparison to control animals, with variation in valve gape and oxygen demand when exposed to pile-driving. The common hermit crab exhibited some behavioural changes also, but this was not significant (Roberts *et al.*, 2016). The limited amount of research into the impact of pile-driving may have on marine invertebrates is worrying due to the important roles that invertebrates play in the oceans. Ecologically, they play a vital role in marine ecosystem functioning as they are the most abundant prey organisms, some also filter feed sediment from the water column while others act as bioturbators in the seabed. In the commercial and social sense, many marine invertebrates are also important because they are a key source of food for people around the world. More

research is needed into the impact that pile-driving noise may be having on invertebrates which are key to marine ecosystems.

The aim was therefore to improve our understanding of the impact of pile-driving noise on marine invertebrates by conducting two carefully controlled laboratory-based studies. The common shore crab (*Carcinus maenas*) was chosen as the experimental species for several reasons. Previously, two studies demonstrated that playback of the noise of commercial shipping has both physiological and behavioural effects on the common shore crab (Wale *et al*., 2013a, 2013b), demonstrating this species to be sensitive to noise playback. The common shore crab is commonly found in harbours where pile-driving is often undertaken during construction; thus understanding the impact of pile-driving noise playback in the laboratory could give an indication of their response in the environment. Furthermore, the common shore crab has an important ecological role in coastal communities and associated food webs, and is relatively abundant along the British coastline, thus use in research should not pose any threat to wild populations. The shore crabs is also hardy for use in the laboratory, being tolerant to changes in salinity and temperature due to its life history on the rocky shore, including in rock pools (Lewis, 2010). The common shore crab provides a valuable experimental model system for marine decapods more generally, and so helps better understand the effect of pile-driving on other decapods, including commercially important species such as the edible crab (*Cancer pagurus*).

The two complementary studies examined the effect of pile-driving noise playback on the physiology (oxygen consumption and haemolymph parameters) and behaviour (feeding, emergence, immobility) of the crabs. Both these experiments were carried out in laboratory-based tanks at the University of Exeter with the use of an underwater loudspeaker for playback of recorded pile-driving noise, comparing responses during noise exposure to those with a control of recorded ambient coastal noise. This study tested the hypotheses that there would be significant differences in oxygen consumption, haemolymph parameters and behavioural responses when crabs were exposed to pile-driving noise playback in comparison to ambient playback.

2.2 Materials and Methods

2.2.1 Experimental Animals

Pre-moult male common shore crabs (*Carcinus maenas)* were obtained on 2 and 16 March 2017 from a local fisherman at Exmouth Marina, having been trapped using guttering the same day on the Exe Estuary mudflats. The crabs were then transported to the University of Exeter's Aquatic Resources Centre in a large tank and kept damp with wet towels. Once there, ectoparasites were removed from their carapaces. The crabs were then transferred to a holding tank (80 x 65 x 75 cm) enriched with an area to shelter in and connected to an artificial salt water recirculation system. In the holding tank, the temperature was kept at 16°C, pH at 8.1 ± 0.2 (mean ± SE), salinity at 33.1 PSU, with a photoperiod of 14:10 (day:night). Crabs for both experiments (n = 70) weighed 48.1 ± 2.3 g (mean ± SE) and were starved for 4 days before experimentation.

2.2.2 Physiological Experimental Design

A subset of the largest shore crabs was removed from the holding tank on the day after each collection (3 and 17 March). Mass (g ± 0.02; measured using an OHAUS AV412 balance), water displacement (ml; measured by placing a crab in a full container of saltwater and measuring the water displaced), condition (number of legs and claws) and colour morphology (red or green) were noted for each crab. Green shore crabs (n = 23) weighed 62.5 ± 4.3 g (mean ± SE) and displaced 53.7 ± 3.9 ml (mean ± SE). Red shore crabs (n = 19) weighed 53.7 ± 2.6 g (mean ± SE) and displaced 43.2 ± 2.3 ml (mean ± SE). The different colour morphs were analysed independently of each other due to differing morphologies (Reid *et al.*, 1997; Lewis, 2010).

Each experimental run was set up with four large experimental tanks (76.5 x 56.5 x 41 cm) which were each connected to a sump to provide additional water volume (Fig. 2.1).

Figure 2.1. An experimental tank (top) and sump (bottom) for the crab physiology experiment.

An Aqua-30 underwater loudspeaker (DNH, 30 W, frequency range: 80–20,000 Hz) was placed in the middle of each experimental tank, on top of foam to reduce friction vibrations. The loudspeaker was connected to an 18 W amplifier (Kemo Electronic GmbH, frequency response: 40-20,000 Hz) with a potentiometer (Omeg Ltd) set to minimum resistance, all powered by a 12 V battery, and an MP3 player (Bush, frequency range: 20-20,000 Hz). In each tank, surrounding the underwater loudspeaker were six 3.2 L plastic airtight containers each housing a crab for the duration of the experiment (Fig. 2.2).

Figure 2.2 Six 3.2 L plastic containers with water supply in an experimental tank for the crab physiology experiment.

The containers were placed equidistant from each other and 6 cm from the loudspeaker (Fig. 2.3).

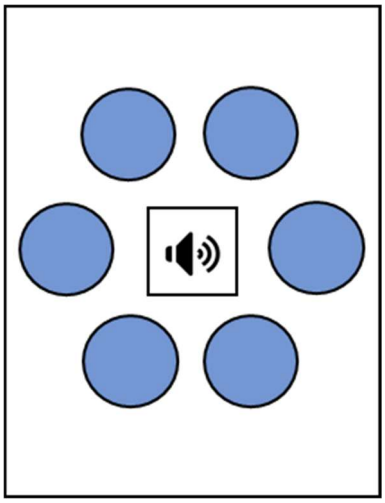

Figure 2.3. Positioning of the six 3.2 L containers, equidistantly spaced around the underwater loudspeaker in the crab physiology experiment.

Crabs were shielded from viewing other crabs in the tank and starved throughout to standardise their condition. Water was pumped directly into the containers which overflowed into the main tank and then was drained into the sump for recirculation. The water was oxygenated by air stones in the sump to reduce background noise in the main experimental tank. The experimental tanks were seated on foam to reduce noise transfer to the other experimental tanks on the workbench. The water temperature was kept on average at 15.1 °C ± 0.3, pH at 8.1 ± 0.003, salinity at 33.45 PSU ± 0.06 and a photoperiod of 12:12 (day:night). Ammonia levels were kept below 0.01 mg/L throughout the experiment.

An ambient treatment and a pile-driving treatment were used for playback in the tanks. Two ten-min ambient treatment and two ten-min pile-driving treatment WAV tracks for playback were mixed using Audacity 2.1 (The Audacity Team; https://www.audacityteam.org). For the pile-driving treatment tracks, pile-driving noise was mixed over the top of an ambient track. The original ambient sound tracks used were recorded at the ports of Gravesend, UK (51°26'42" N, 0°22'37" E) and Plymouth, UK (50°21'33" N, 04°07'26" W), see Wale *et al.* (2013a) for further details. The original pile-driving track was recorded at Blyth, UK (55°08'46" N, 01°25'15" W) at approximately 120 m from a pile-driver; see Poulton *et al.*

(2016) for further details. Each of the tracks for each treatment were played on a loop for the duration of the experiment.

After mass, water displacement, condition and colour morphology were noted for each crab, they were placed into their individual experimental containers. Crabs were distributed evenly, with three green crabs and three red crabs in each of the experimental tanks. They were left to acclimate to the set up for three days with no noise playback. Four experimental tanks were used in each experimental run. Two experimental runs were undertaken on the 6 and 20 March, each experimental run was with half the experimental tanks exposed to the ambient treatment (n = 4) and the other half exposed to the pile-driving treatment (n = 4). Each treatment had an initial period of 15 min with the containers left open. After 15 min, the water flow was cut off to the containers, an oxygen concentration (μM) reading was taken with a FireSting O_2 oxygen probe (Pyro Science) and the containers were sealed to be air/watertight. After 75-85 min of continuous playback of either the ambient treatment or the pile-driving treatment, the containers were opened, and another oxygen consumption reading was taken. As a control, oxygen concentration (μM) was measured before and after the experiment with three containers of experimental tank water for each of the treatments to account for the oxygen consumption of bacteria in each tank. Oxygen consumption rate (μM/hour) was calculated for the crabs, correcting for body mass, time spent sealed in container and bacterial oxygen consumption.

After the last oxygen reading in each tank, approximately 200 μl of haemolymph was removed using a 1 ml syringe with a 23-gauge needle from each crab from the arthrodial membrane on the coxa of the fourth pair of walking legs. Haemolymph samples were kept on ice prior to measuring pH using a HI8314 membrane pH meter (Hanna instruments). The remaining haemolymph was frozen for analysis of glucose and protein content at a later date.

Before measurement of glucose concentration, the haemolymph was thawed and deproteinated in 1:1 0.6 M perchloric acid, centrifuged at 10,000 rpm for 5 min and the supernatant removed (following Webster, 1996). The supernatant was diluted 1:9 with PBS (Sigma-Aldrich) due to the high concentration of glucose in the haemolymph. Glucose concentration of the samples and glucose standards (Sigma-Aldrich) were then measured using the glucose oxidase method (Webster, 1996) by use of a Glucose Assay Kit (Sigma-Aldrich). This was scaled

down for use on a 96 well microplate and the samples run on a TECAN infinite M200 PRO microplate reader at 540 nm.

Protein concentration was determined for the remaining haemolymph samples after thawing by use of a modified method of Bradford (1976). Samples of haemolymph were diluted 1:40 in PBS (Sigma-Aldrich) due to the high concentration of protein in the haemolymph. The samples and prepared BSA protein standard (Sigma-Aldrich) were then plated on to a 96 well plate, incubated with a protein assay reagent (Bio-Rad) and the samples run on a TECAN infinite M200 PRO microplate reader at 595 nm.

2.2.3 Behavioural Experimental Design

The experimental setup consisted of a tank (60 x 30 x 30 cm) made up of three sections (Fig. 2.4). Section 1 was the smaller area (375 cm^2), furthest from the loudspeaker and the location at which the crab started the trial. This was separated from Section 2 by a tank divider that was raised up to reveal an opening (15 x 6 cm) at the start of the trial. Section 2 was the larger and more open area (1,020 cm^2), containing three circular dishes (6 cm diameter) which were fixed 25 cm from the opening connecting Section 2 to 1. During the trial, these dishes had a rectangular 1.25 cm^2 piece of blue mussel (*Mytilus edulis)* muscle tissue in one and two similarly sized and coloured pieces of elastic band (pseudo-food) in the other two. These were randomly allocated in the dishes in each trial. Behind the next tank divider was Section 3 where an Aqua-30 underwater loudspeaker was positioned upright and placed on foam to prevent direct contact with the tank. Section 3 was both inaccessible and visually obscured from the crab. The underwater loudspeaker was connected to the same amplifier, potentiometer and MP3 player set-up as used for the physiological experiment detailed above. A GoPro Hero3 video camera, was positioned above the experimental tank to record the trial without interference by human presence. The tank was cleaned with ethanol and saltwater replaced after each trial to remove olfactory stimuli from previous crabs.

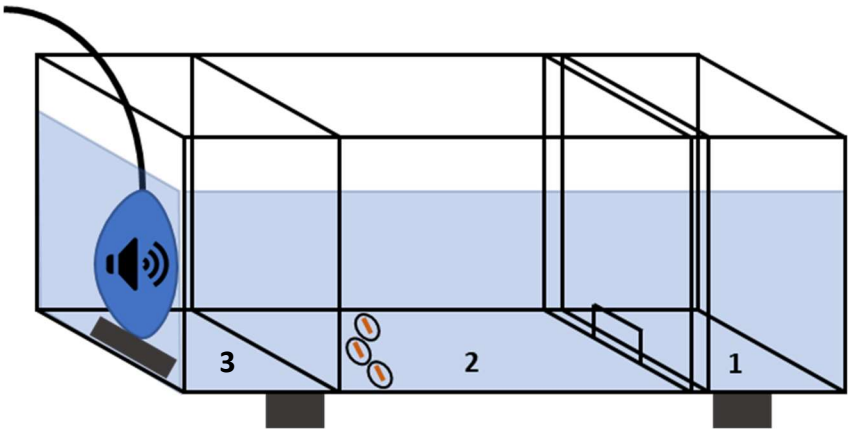

Figure 2.4. Behavioural experimental tank setup, with Section 1, 2 & 3, an underwater loudspeaker (sectioned off) and positioning of both mussel tissue and elastic bands.

An ambient treatment and a pile-driving treatment were used for playback in the experimental tank. Two sets of two different types of ten min playback treatment WAV tracks were mixed for the experiment using Audacity 2.1. Each set was made up of an initial 5-min ambient track for acclimation followed by a further 5-min of either an ambient track (ambient treatment) or a pile-driving track (pile-driving treatment) when the trial began. For the pile-driving tracks, pile-driving noise was mixed over the top of an ambient track. All tracks were made from the same original recordings as detailed in the physiological experiment above.

For each trial, a crab was collected at random from the main holding tank (n = 28; body mass = 32.4 ± 1.5 g, mean ± SE), placed in Section 1 of the experimental tank and given 5 min to acclimatise to the new surrounding whilst the 10 min ambient treatment (n = 11) or pile-driving treatment (n = 17) was started. At the 5 min mark (when the playback track either continues with ambient sound or starts pile-driving noise) the tank divider between Sections 1 and 2 was simultaneously raised to allow crab movement into Section 2 through the rectangular opening if it chose to.

Post-trial videos were analysed for different measures, with crabs excluded from analysis if they climbed out of the water or were inactive during the initial 5 min acclimation (n = 20). Measures recorded included time spent immobile (still for longer than 3 s), whether they emerged from Section 1, time taken to emerge from Section 1, time spent in each section, number of changes between sections, whether blue mussel tissue was eaten, time taken to fully consume mussel tissue and whether they interacted with the elastic bands.

2.2.5 Statistical Analysis

Statistical analyses were carried out using SPSS Statistics 24 (IBM). Chi-square tests, t-tests, ANOVAs, ANCOVAs and Pearson's product moment correlations were used to compare data in the different treatments. Significance was assessed with an alpha-value of less than 0.05. Tukey's HSD was used to test for significance in ANOVAs between four different treatments (ambient-green crabs, pile-driving-green crabs, ambient-red crabs and pile-driving-red crabs). Significance was assessed with an alpha-value of 0.05.

2.2.6 Acoustic Analysis

Waveforms were generated in Audacity 2.1 from the original tracks used for playback and from in-tank recordings (Fig. 2.5).

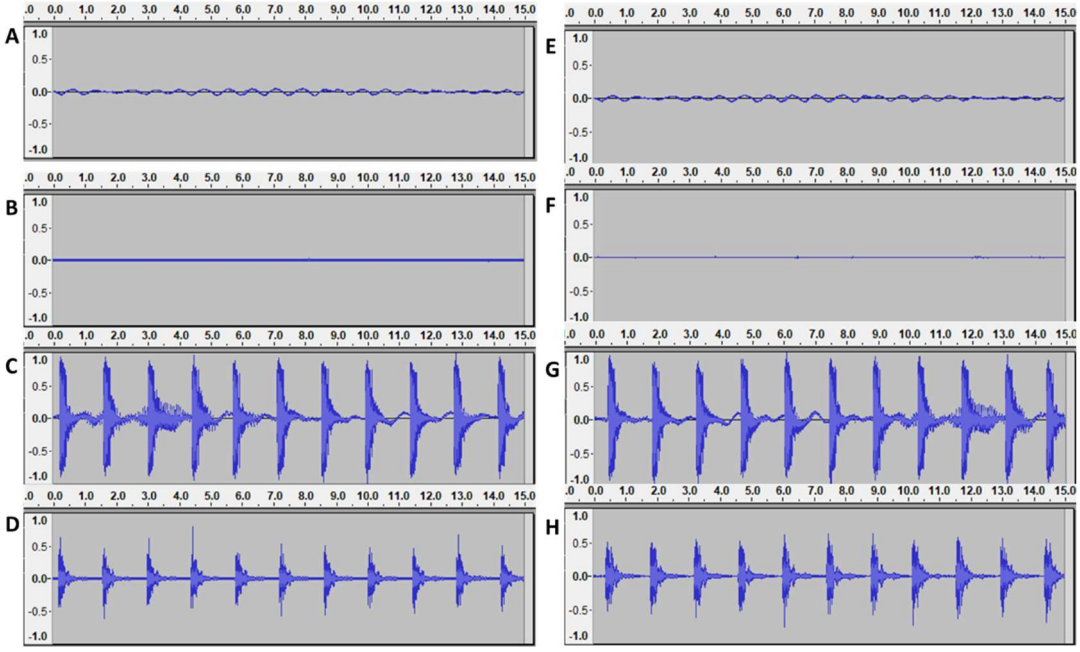

Figure 2.5. Examples of waveforms (15 s long) for A) original ambient treatment track, B) recording of ambient treatment playback in the physiology tank, C) original pile-driving treatment track, D) recording of pile-driving treatment playback in the physiology tank, E) original ambient treatment track, F) recording of ambient treatment playback in the behavioural tank, G) original pile-driving treatment track, and H) recording of pile-driving treatment playback in the behavioural tank.

For both the physiological and behavioural study, a recording of both the ambient and pile-driving treatments from the experimental tanks were analysed in MATLAB (V. 2013a) using paPAM (Nedelec *et al.*, 2016). Illustrative spectrograms (Fig. 2.6) were generated for 0-3,000 Hz, the most relevant frequency levels for crustaceans (Lovell *et al.*, 2005). These were calculated

using a Hamming evaluation window, sample rate window length and 50% overlap over 10 s.

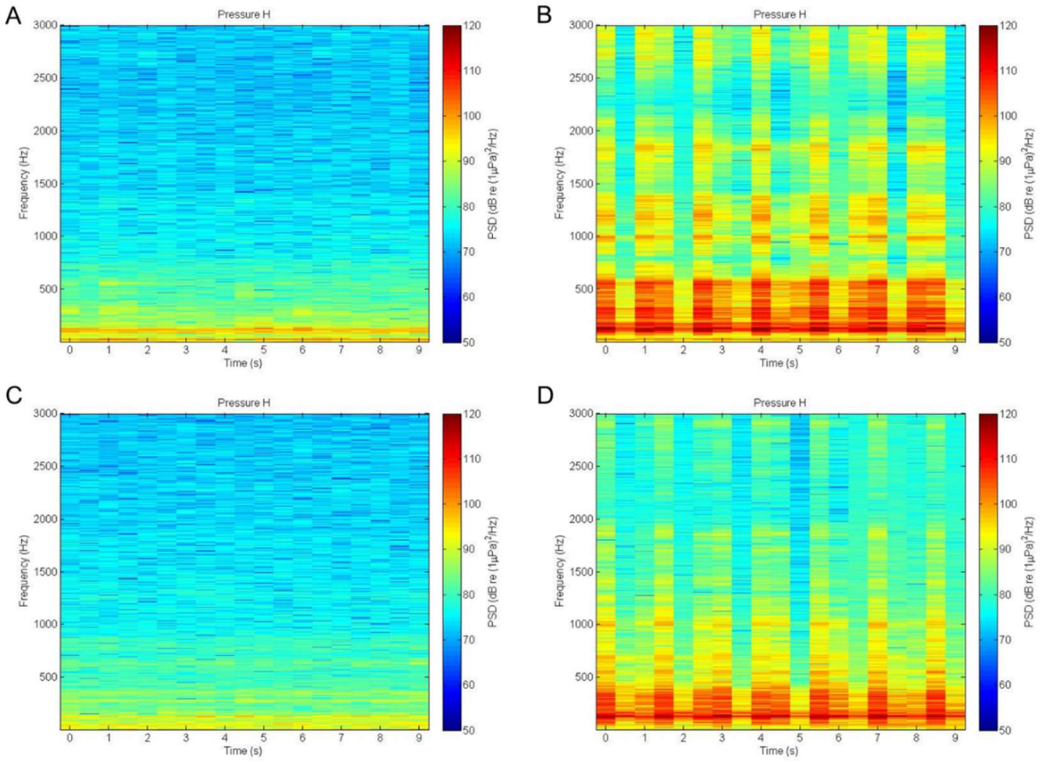

Figure 2.6. Illustrative spectrograms of A) ambient treatment playback in the physiology tank, B) pile-driving treatment playback in the physiology tank, C) ambient treatment playback in the behavioural tank, and D) pile-driving treatment playback in the behavioural tank.

2.3 Results

2.3.1 Crab Physiology

2.3.1.1 Oxygen Consumption

For green crabs, a significant positive relationship was found between mass and oxygen consumption rate for both the ambient treatment (Pearson's product-moment correlation: r = 0.878, n = 11, p < 0.01; Fig. 2.7) and the pile-driving treatment (r = 0.853, n = 12, p < 0.01; Fig. 2.7).

There was no significant difference in oxygen consumption rate between the two treatments (ANCOVA: $F_{1,20}$ = 0.149, p = 0.703; Fig. 2.7), whilst adjusting for crab mass ($F_{1,20}$ = 0.746, p < 0.01).

For red crabs, a significant positive relationship was found between mass and oxygen consumption rate for the pile-driving treatment (Pearson's product-moment correlation: r = 0.784, n = 11, p < 0.01; Fig. 2.7); however, there was no significant relationship for the ambient treatment (r = 0.099, n = 8, p = 0.816; Fig. 2.7).

There was no significant difference in oxygen consumption rate between the two treatments (ANCOVA: $F_{1,16}$ = 2.192, p = 0.158; Fig. 2.7), whilst adjusting for crab mass ($F_{1,16}$ = 0.364, p < 0.01).

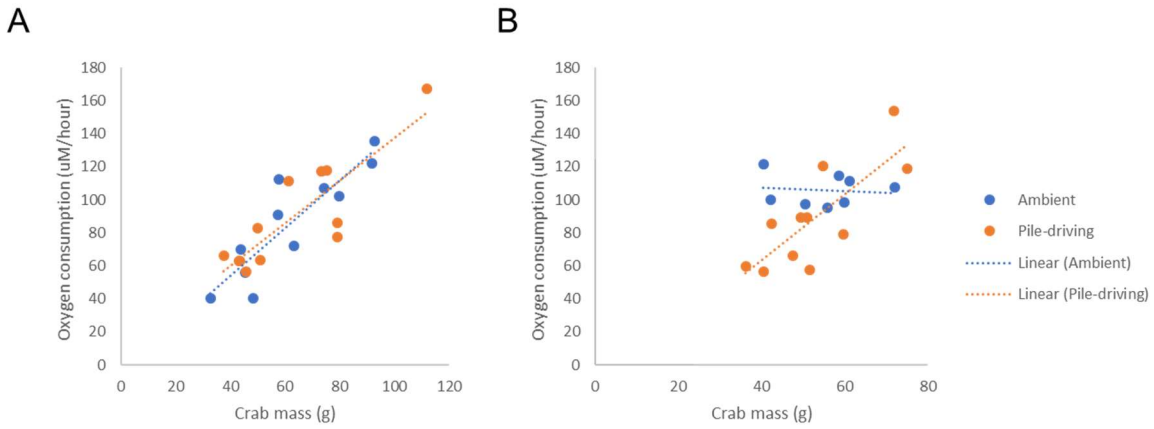

Figure 2.7. A) The relationship between oxygen consumption rate and mass of green crabs exposed to either the ambient treatment (n = 11) or pile-driving treatment (n = 12). B) The relationship between oxygen consumption rate and mass of red crabs exposed to either the ambient treatment (n = 8) or the pile-driving treatment (n = 11).

2.3.1.2 Haemolymph Measurements

There was no significant difference between noise treatments for green crabs (Tukey HSD: p = 0.960) or for red crabs (p = 0.625). There was a significant difference in haemolymph glucose concentration between the different treatments (one-way ANOVA: $F_{3,37}$ = 10.758, p < 0.01; Fig. 2.8). This significant difference was found between colour morphologies. Ambient-red crab glucose concentration was significantly greater in comparison to both ambient-green crab (p = 0.036) and pile-driving-green crab glucose concentration (p = 0.01). Pile-driving-red crab glucose concentration was also significantly greater in comparison to both ambient-green crabs (p = 0.01) and pile-driving green crabs (p < 0.01).

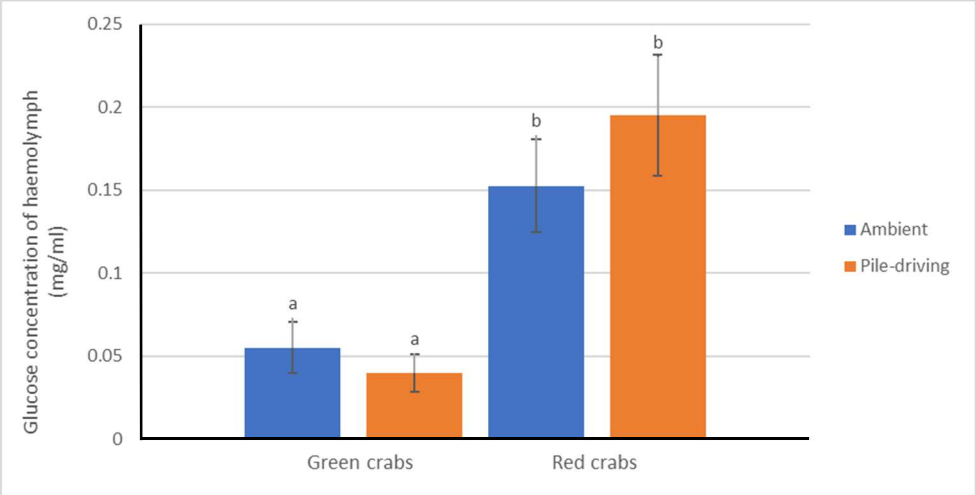

Figure 2.8. Mean ± SE glucose concentration of green and red crab haemolymph when exposed to either the ambient treatment (green crabs, n = 9, red crabs, n = 8) or the pile-driving treatment (green crabs, n = 12, red crabs, n = 10). Letters denote significantly different treatments (Tukey HSD).

There was no significant difference between noise treatments for green crabs (Tukey HSD: p = 0.959) or for red crabs (p = 1.00). There was a significant difference in haemolymph protein concentration between the different treatments (one-way ANOVA: $F_{3,37}$ = 4.407, p = 0.01; Fig. 2.9). This significant difference was found between colour morphologies. Protein concentration was only significantly greater in pile-driving-red crabs in comparison to pile-driving-green crabs (p = 0.03).

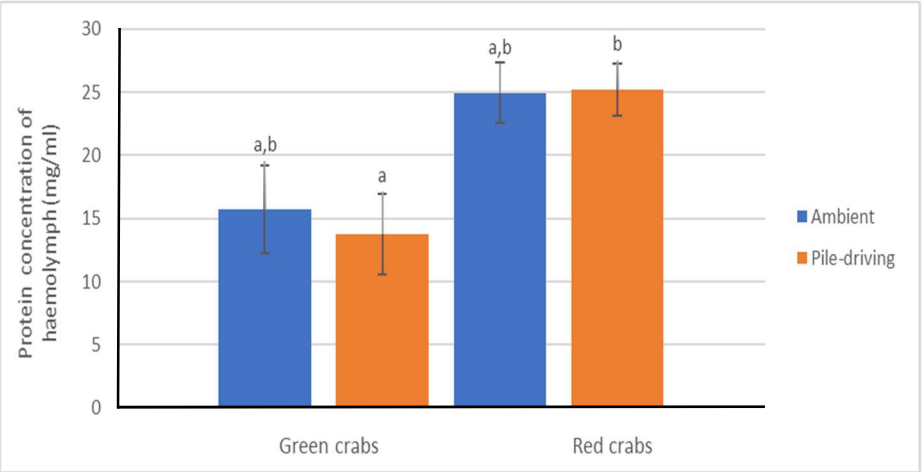

Figure 2.9. Mean ± SE protein concentration of green and red crab haemolymph when exposed to either the ambient treatment (green crabs, n = 9, red crabs, n = 8) or the pile-driving treatment (green crabs, n = 12, red crabs, n = 10). Letters denote significantly different treatments (Tukey HSD).

There was not a significant difference in haemolymph pH between the different treatments (one-way ANOVA: $F_{3,35} = 0.268$, $p = 0.848$; Fig. 2.10).

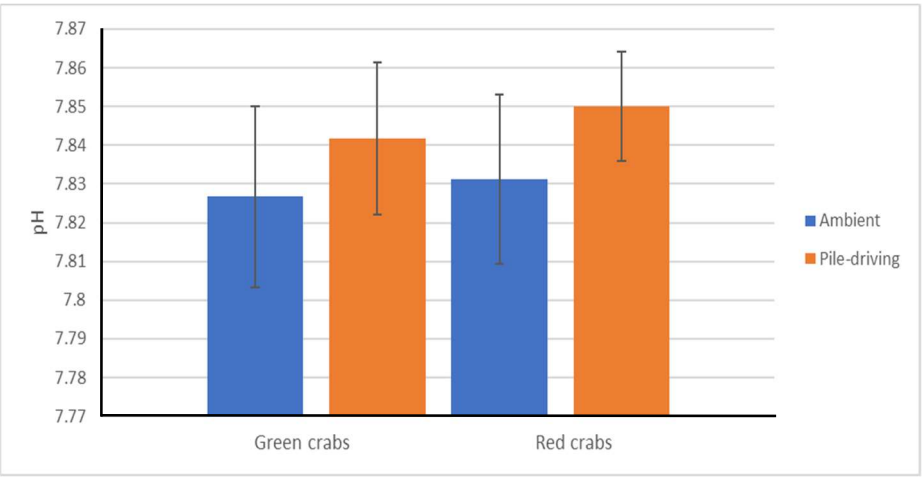

Figure 2.10. Mean ± SE pH of green and red crabs exposed to either the ambient treatment (green crabs, n = 9, red crabs, n = 8) or the pile-driving treatment (green crabs, n = 12, red crabs, n = 10).

2.3.2 Crab Behaviour

The treatment had a significant effect on whether crabs consumed mussel tissue or not (Chi-square test: $X^2_1 = 5.812$, n = 28, p = 0.016). Crabs were more likely to consume the mussel tissue in the ambient treatment than in the pile-driving treatment (Fig. 2.11)

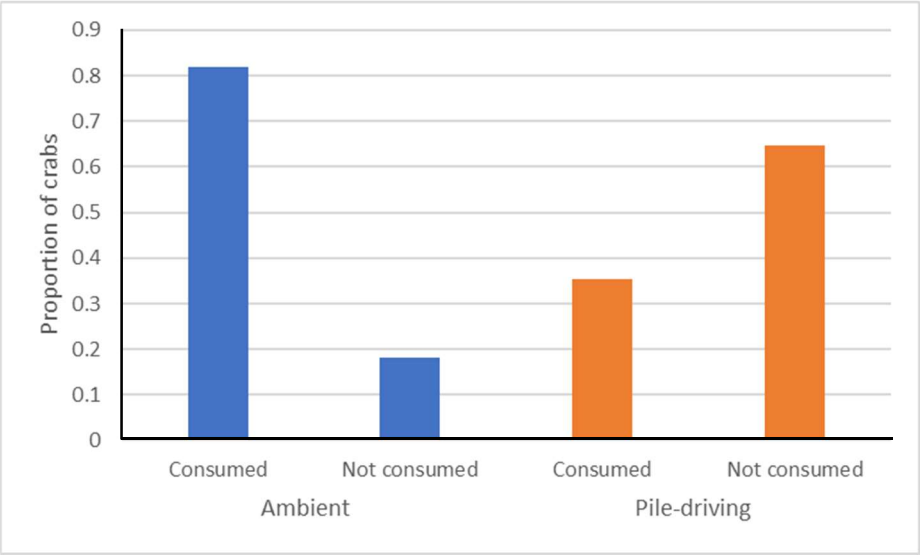

Figure 2.11. Proportion of crabs that consumed the mussel tissue when exposed to either the ambient treatment (n = 11) or the pile-driving treatment (n = 17).

For crabs that did fully consume the mussel tissue, the mean time taken was not significantly different between treatments (Independent t-test: $t_{13} = 0.354$, p = 0.729; Fig. 2.12).

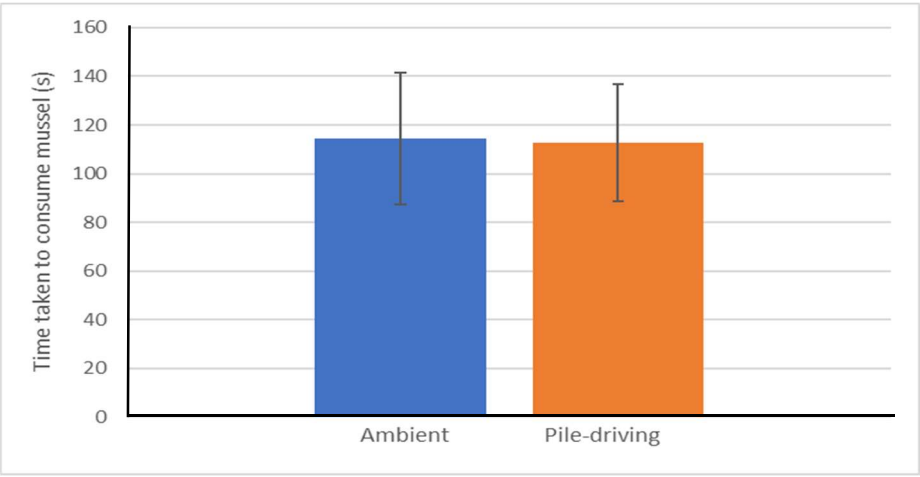

Figure 2.12. Mean ± SE time taken for crabs to fully consume mussel tissue (only for crabs that consumed mussel tissue during the trial) when exposed to either the ambient treatment (n = 9) or the pile-driving treatment (n = 11).

The treatment did not have a significant effect on whether crabs interacted with the pseudo food (Chi-square test: $X^2_1 = 0.225$, n = 28, p = 0.636; Fig. 2.13).

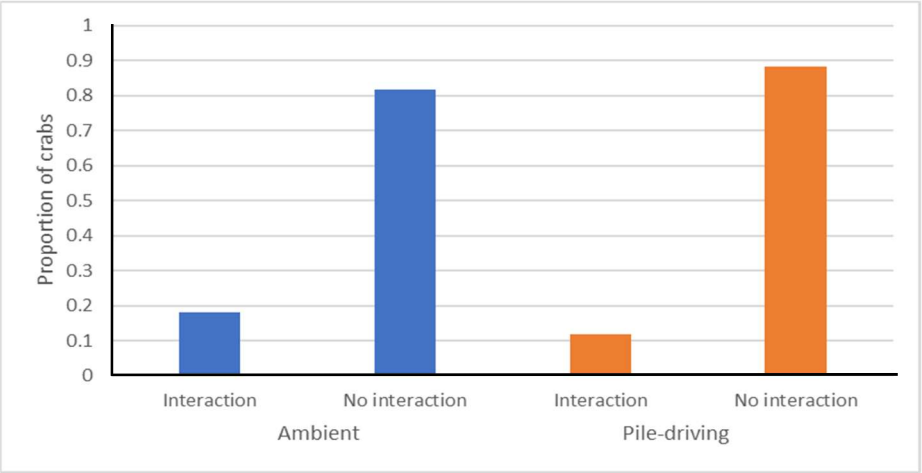

Figure 2.13. Proportion of crabs that interacted or had no interaction with an elastic band (pseudo-food) when exposed to either the ambient treatment (n = 11) or the pile-driving treatment (n = 17).

The mean time crabs were immobile was significantly greater in the pile-driving treatment in comparison to the ambient treatment (Independent t-test: $t_{26} = 2.063$, p = 0.049; Fig. 2.14).

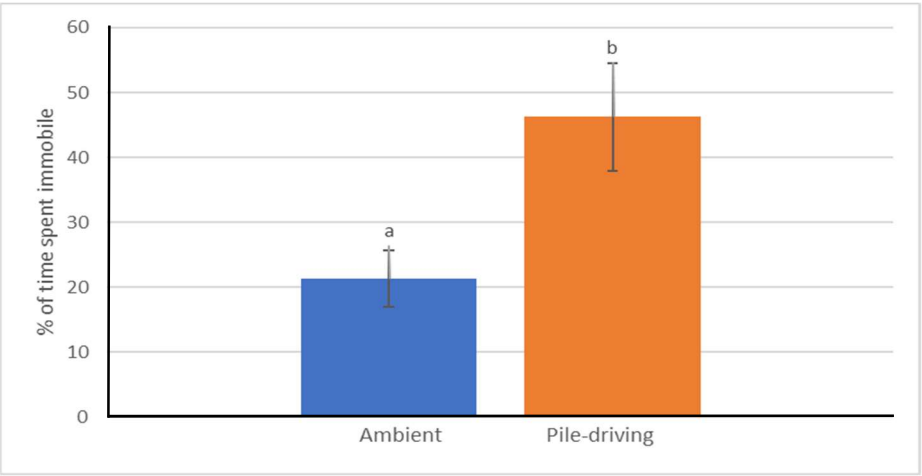

Figure 2.14. Mean ± SE percentage of time crabs were immobile (still for longer than three s) when exposed to either the ambient treatment (n = 11) or the pile-driving treatment (n = 17). Letters denote significantly different treatments.

The treatment did not have a significant effect on whether crabs emerged from Section 1 or not (Chi-square test: $X^2_1 = 0.949$, n = 28, p = 0.33; Fig. 2.15).

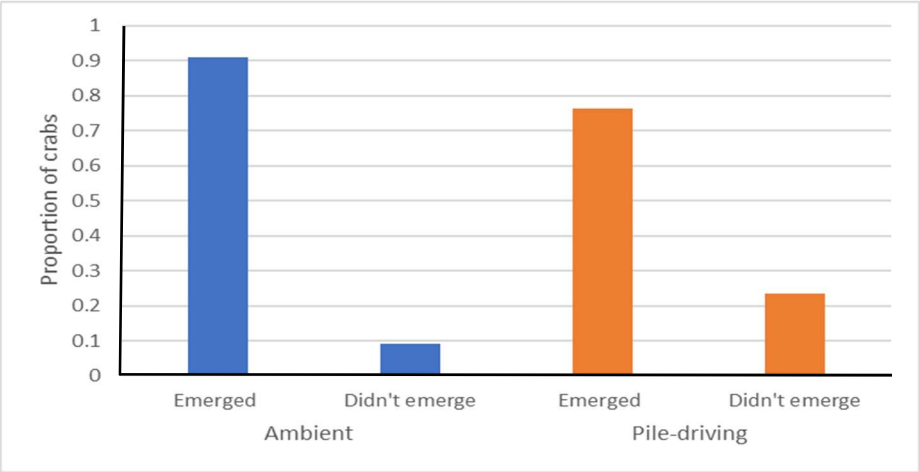

Figure 2.15. Proportion of crabs that emerged from Section 1 (starting area) when exposed to either the ambient treatment (n = 11) or the pile-driving treatment (n = 17).

For crabs that did emerge from Section 1, there was no significant difference between treatments in the time taken to emerge (Independent t-test: $t_{21} = 0.682$, $p = 0.504$; Fig. 2.16).

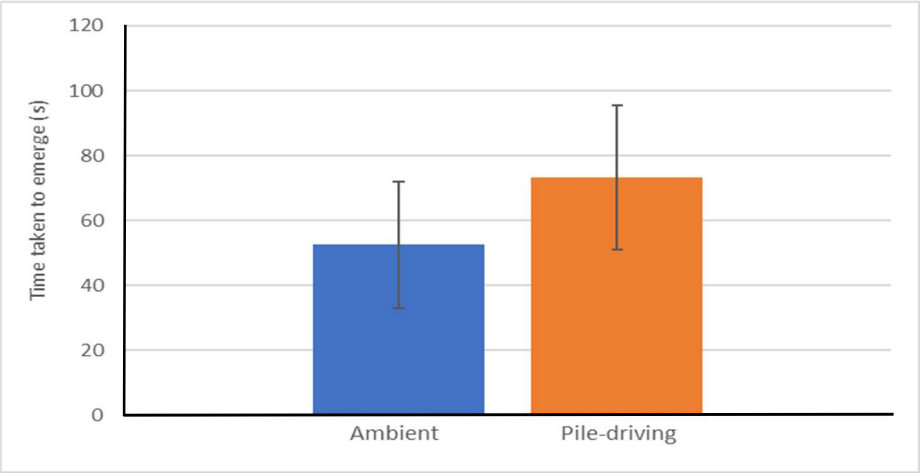

Figure 2.16. Mean ± SE time taken for crabs to emerge from Section 1 (starting area) for individuals that emerged when exposed to either the ambient treatment (n = 10) or the pile-driving treatment (n = 13).

The mean percentage of time crabs spent in Section 2 was significantly greater in the ambient treatment in comparison to the pile-driving treatment (Independent t-test: $t_{26} = 2.906$, $p < 0.01$; Fig. 2.17).

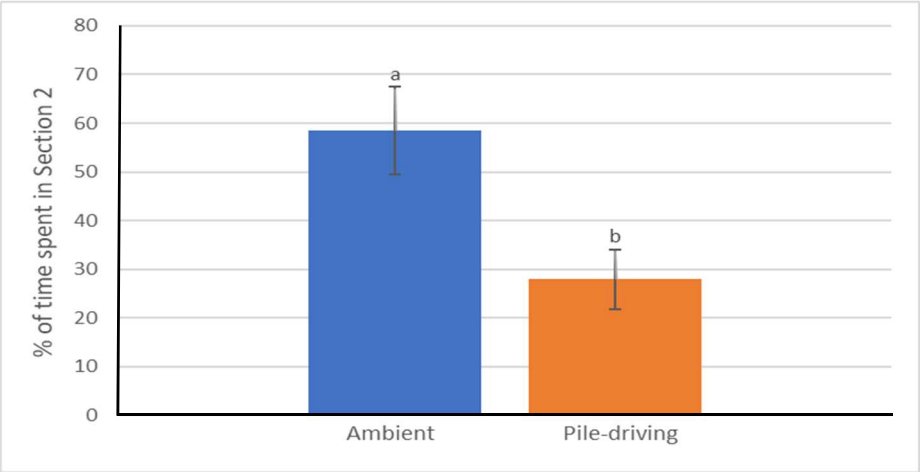

Figure 2.17. Mean ± SE percentage of time crabs spent in Section 2 when exposed to either the ambient treatment (n = 11) or the pile-driving treatment (n = 17). Letters denote significantly different treatments.

2.4 Discussion

The increased input of anthropogenic noise into the ocean is a concern for marine fauna. The aim of these two experiments was to explore the effect that simulated pile-driving noise may have on the physiology and behaviour of a marine invertebrate, in this case the common shore crab. Playback of pile-driving noise in experimental tanks did not have a significant effect on the measured aspects of crab physiology. However, playback of pile-driving noise did have a significant effect in altering the behaviour of the crabs in a feeding study. Previous research into the physiological responses of marine species to pile-driving noise has primarily focused on fish. Physiological responses observed in response to both pile-driving noise playback and simulated pile-driving include increased ventilation, barotrauma injuries and in some cases mortalities (Casper *et al.*, 2012, 2013a, 2013b; Poulton *et al.*, 2016). Relatively little research has explored the effect of pile-driving noise on invertebrates; and this is the first study to explore the physiological response of a decapod invertebrate (*C. maenas*) to pile-driving noise playback.

2.4.1 Physiological Responses

A positive relationship was observed for oxygen consumption rate against mass for both the green crab noise treatments (Fig. 2.7) and the red crab pile-driving treatment (Fig. 2.7). However, there was no significant positive relationship for the red crab ambient treatment (Fig. 2.7), which is likely due to a low sample size (n = 8). This positive relationship between oxygen consumption rate against mass was expected, with previous research on common shore crabs showing an increase in oxygen consumption with increased mass (Wale *et al.*, 2013b). However, there was no significant difference in oxygen consumption between the ambient and pile-driving treatments in either crab colour morphs. It was predicted that pile-driving noise playback would cause an increase in oxygen consumption, similar to the stress response in fish. In fish, increases in ventilation and oxygen consumption rate occur as a result of stress, indicative of an increased metabolic rate (Wendelaar Bonga, 1997). In one study European seabass were found to have increased ventilation rate when exposed to pile-driving noise (Purser *et al.*, 2016), and in another European eels (*Anguilla anguilla*) had elevated ventilation and metabolic rates with ship noise playback (Simpson *et al.,* 2015); both studies indicating a stress response to noise. There is limited research into the effect of

noise on the physiology of marine invertebrates, but one previous study into the physiological effects of continuous ship noise on common shore crabs (albeit with a different anthropogenic noise source) found that playback of ship noise in experimental tanks caused an increase in oxygen consumption (Wale *et al.*, 2013b). The fact that the crabs did not seem to be stressed physiologically in the current study with exposure to pile-driving noise playback is surprising considering these previous research studies.

There was no significant difference in glucose concentration between noise treatments for the two different colour morphologies of crabs (Fig. 2.8). Glucose was expected to be higher in the pile-driving treatment, due to the hyperglycaemic stress response of crustaceans. When exposed to an environmental stressor, crustaceans have been shown to release the crustacean Hyperglycaemic Hormone (cHH) from a sinus gland in their eyestalks. This hormone release results in elevated levels of glucose in their haemolymph (Webster, 1996; Lorenzon, 2005) and allows the crabs to control the levels of glucose in their haemolymph (Lüschen *et al.,* 1993). Previous research on the European spiny lobster (*Palinurus elephas*) using a different anthropogenic noise source showed that boat noise playback in experimental tanks resulted in significantly elevated glucose levels (Filiciotto *et al.*, 2014). When exposed to a stressor, *Cancer pagurus* took over 45 min for glucose concentration to rise significantly in the haemolymph in comparison to the control (Webster, 1996). This delay means it is possible that glucose could have risen for the pile-driving treatment in the current study if the crabs had been left for longer before sampling. Interestingly, glucose concentration was significantly greater in both red crab noise treatments in comparison to the green crab noise treatments (Fig. 2.8) and is most likely attributed to their different life histories (Lewis, 2010). Green crabs are often found on the intertidal zone, and are thought to be more tolerant to environmental fluctuations whereas red crabs remain in the subtidal zone with a longer inter-moult stage, resulting ultimately in a stronger and thicker carapace (Reid *et al.*, 1997). Red crabs are less tolerant of environmental fluctuations and may be more likely to be stressed by the experimental water parameters, which could explain the greater glucose concentration in red crab haemolymph.

Protein concentration was not significantly different between noise treatments in both crab morphologies (Fig. 2.9). This was predicted to be different in crabs

exposed to pile-driving noise due to variable protein expression in haemolymph found in crustaceans when exposed to various stressors (Chang, 2005; Fredrick and Ravichandran, 2012; Filiciotto *et al.*, 2014). A previous research study exploring the effect of boat noise on haemolymph protein concentration found that, *P. elephas* had a greater haemolymph protein concentration in the boat noise treatment (Filiciotto *et al.*, 2014). Haemolymph protein concentration was significantly greater for the red crabs in the pile-driving treatment in comparison to the green crabs in the pile-driving treatment. However, this was not significantly different between any of the other protein treatments, which may again be a result of a low sample size (n = 8). This is similar to the results in the glucose study, with the two different morphologies having different life histories (Reid *et al.*, 1997) and thus different physiologies.

The pH of crab haemolymph was not significantly different between the two crab morphologies and noise treatments, even though both pile-driving treatments were on average greater (Fig. 2.10). It was theorised that the pH of the haemolymph could be affected by pile-driving noise as part of a stress response. Previous studies have observed haemolymph pH changes in response to stressors such as emersion and hypoxia (Burnett and Johansen, 1981; Lorenzon *et al.*, 2008).

The fact the crabs did not appear to differ in the measured physiology in response to pile-driving playback is surprising given previous research. The noise playback in the experimental tank of the pile-driving treatment was shown to be different to the ambient treatment as intense pile-driving peaks can be seen in comparison to ambient sound playback on the spectrogram, with intense peaks ranging from 0–3,000 Hz (Fig. 2.6). Frequencies between 100 and 3,000 Hz previously evoked an Auditory Brainstem Response (ABR) in a decapod crustacean, the common prawn (*Palaemon serratus*; Lovell *et al.*, 2005), indicating they can detect sound at the frequencies in the experimental tank.

One possibility is that the crabs are not physiologically stressed by this sound level or type of noise playback. This could be due to the estuarine area they were collected from where potentially they would be used to greater environmental variation in temperature, salinity and wave action and therefore could be more resilient to stressors. However, it is also possible that the ambient sound was loud enough to cause a physiological response itself and which could not be

differentiated from the physiological response to pile-driving noise due to both crab noise treatments reaching a maximal state of stress. This could also be said for the experimental setup, which despite the attempt to acclimatise the crabs by housing them in the setup for a few days beforehand and reducing background noise, the crabs may still be stressed by the setup. The experimental procedure involved removing water flow to the experimental containers, which could also cause a maximal stress response in both crab treatments before starting the experimental trial.

Another possibility for the lack of a detectable physiological response is that the crabs may be more sensitive to sound through vibrations in sediment since they detect sound with chordotonal organs in their joints (Roberts and Elliott, 2017). To reduce the vibrations in the tank from the loudspeaker, it was placed on top of foam. This may have reduced the transfer of pile-driving noise to the ground of the experimental tank and consequently reduced the capability of the crabs to detect the noise with a resulting stress response.

As a result of the crabs having been previously starved after being brought in to the research facility, they may have reduced activity to conserve energy and thus reduced the likelihood of detecting a response to any potential stressors. This may also explain why the glucose concentration in the haemolymph was similar in both noise treatments having not eaten for several days.

2.4.2 Behavioural Responses

In this study, pile-driving noise playback affected the behaviour of the common shore crab including feeding and immobility.

The noise treatment did not significantly affect whether crabs emerged from Section 1 of the experimental tank during the trial (Fig. 2.15) or the time it took for them to emerge (Fig. 2.16). An effect was predicted as it was expected that they would be able to detect the intense pile-driving noise and potentially recognise it as a threat to avoid. It is possible that having sensed the noise they were then trying to find an area to hide in by exploring or having been in a starved condition that the need for food outweighed hiding behaviour.

Crab feeding behaviour was affected by pile-driving treatment. Crabs were significantly less likely to consume mussel tissue in the pile-driving treatment (Fig. 2.11), but there was no significant difference in the time taken to do so (Fig. 2.12).

The reduced likelihood of feeding could have been due to several factors. The crab may have perceived the pile-driving noise as a threat from a predator and decided that the danger from this outweighed the necessity to scavenge for food. The noise could have equally stressed the crab resulting in a disinterest in scavenging. It is also possible that the crabs could have been distracted and were focussing on identifying the noise rather than trying to scavenge. Previous research into ship noise supports this idea of a perceived threat whilst scavenging for food, where in response to ship noise playback common shore crabs were more likely to be disrupted during feeding in comparison to ambient sound playback (Wale *et al.,* 2013a). Another study on a population of wild Mediterranean damselfish (*Chromis chromis*) in an MPA found a similar response, with lower feeding frequencies corresponding with greater boat traffic volume (Bracciali *et al.*, 2012).

Noise treatment did not affect whether crabs interacted with the pseudo-food elastic bands (Fig. 2.13). This indicates that noise playback did not cause crabs to mistake mussel tissue shaped elastic bands for food. This is likely due to crab olfaction being sensitive to odours when scavenging (Kaiser *et al.,* 1993) and easily being able to recognise real food.

A greater amount of time was spent immobile in the pile-driving treatment compared to the ambient treatment (Fig. 2.14). Immobility is an antipredation method and is used by many species to avoid detection by predators. This is likely the reason crabs displayed freezing behaviour to avoid the perceived predator threat of the pile-driving playback. Freezing behaviour has also previously been observed in marine fish; for example, a study on Atlantic cod in large net pens demonstrated a freezing response to the onset of pile-driving noise (Mueller-Blenkle *et al.*, 2010).

Crabs spent longer in Section 2 in the ambient treatment compared to the pile-driving treatment (Fig. 2.17). Section 2 was nearer the noise source but was also a much larger, open and brighter space in comparison to Section 1 where the crabs started the trial. This may indicate to the crabs that they could potentially be more at risk of predation by trying to scavenge in the larger arena. Even with the incentive of food, the pile-driving playback seems to have deterred crabs from spending as much time in Section 2.

2.4.3 General Conclusions

This study has shed some light on some of the physiological and behavioural responses of the common shore crab to pile-driving noise playback. The playback in the experimental tanks was different for the ambient treatment and pile-driving treatment (Fig. 2.6) and was in the known frequency range of detection for a decapod invertebrate (Lovell *et al.*, 2005). The common shore crabs did not appear to be physiologically affected by pile-driving noise for oxygen consumption, pH, glucose concentration and protein concentration. However, crab behaviour appears to have been altered in several ways in response to pile-driving playback in the feeding experiment. In the pile-driving treatment they were significantly less likely to feed on blue mussel tissue and spend time in Section 2, where the food was located. If this occurred over a longer period in a real-world situation, it could have potential fitness costs due to a lack of nutrition. Crabs were also more likely to be immobile in the pile-driving treatment, which could be viewed as a response to a perceived threat. This could be problematic in the environment when trying to avoid actual predators. These results are similar to the findings of Wale *et al.* (2013a) into the effect of boat noise on common shore crabs, which resulted in changes in foraging and antipredator behaviours.

Better understanding of the effects of pile-driving noise on marine invertebrates is important to best mitigate impacts in the oceans. Invertebrates are ecologically important for ecosystem functioning, socially for nutrition and commercially for fisheries. If there is a potential adverse effect on invertebrates during pile-driving, including behavioural changes that impact on feeding or antipredation, consideration must be taken for what can be done to mitigate such impacts, whether through temporal, spatial or technological and mechanical reductions in noise.

The common shore crab was demonstrated to be a valuable tool for research in the laboratory, showing some interesting behavioural responses to pile-driving noise. Behavioural differences could be seen with relatively low sample sizes, helping to reduce the impact on natural populations when obtained for research. Using the common shore crab was also beneficial as they are cheap, abundant, have an important role in the food chain and can be representative of important commercial decapod species including the edible crab.

This study also highlights the significant differences in haemolymph properties between the two different colour morphs of the common shore crab, demonstrating the importance of physiological studies to study colour morphologies separately.

In conclusion, playback of pile-driving noise did not compromise measured physiology in the common shore crab in this the first experiment, however, it did significantly alter some of their behaviours in the second behavioural experiment. Although difficult to extrapolate to the natural environment, this gives an idea of the problem that pile-driving could potentially cause to wild species. As invertebrates are very important from an ecological and commercial sense, this study demonstrates further why impacts on these species should be considered when undertaking industrial activities such as pile-driving in the ocean.

Chapter 3

The Marine Community-level Response to Pile-driving Noise

3.1 Introduction

Anthropogenic noise in the marine environment is now recognised as a significant threat (Tasker *et al.*, 2010; Gedamke *et al.*, 2016). There have been a multitude of studies exploring the impacts of noise on marine fish (Slabbekoorn *et al.*, 2010), but these tend to focus on either physiology or behaviour of individuals of a given species. Far less research has explored the community-level effects of noise exposure (Mensinger *et al.*, 2016). It is important to study the effects of noise at the community-level in fish because their behaviour will be likely to change with the presence of different species of invertebrates, fish and mammals in the environment.

Laboratory-based marine ecological research can be useful in a variety of ways. In a laboratory setting, close can control can be taken over tank conditions such as water chemistry and light levels, adding to the reliability of the research because extraneous conditions can be kept near constant. However, laboratory research should not be directly extrapolated to wild populations, but it can be a good indicator of what might be expected to occur *in situ*. In toxicological research, the laboratory setting is normally where research is conducted, so that wild populations are not exposed to dangerous toxins. Research in the laboratory often used captive bred species, which can help to reduce pressures on wild populations. Also, physiological research is often carried out in the laboratory due to the need for specialist equipment, which would be difficult to deploy and use *in situ*, especially underwater. This is true for marine anthropogenic noise research; for example, the difficulty in deploying loudspeakers into the environment and keeping the remaining equipment dry. However, studying the community-level response is near impossible in the laboratory.

Field based marine ecological research is useful in a great deal of cases but does have limitations. It is logistically more complicated, involving having to contend with the ocean and sometimes unfavourable weather conditions. This can make research difficult and can impact the collection and validity of the data. The cost of research is another consideration, with laboratory work generally being cheaper than fieldwork, due to the need to travel. In marine ecological research

this often means travelling to another country. However, there are benefits to carrying out research *in situ*. Larger marine species, such as marine mammals, cannot be easily housed, making them more suited to research *in situ*. The main advantage of carrying out research *in situ* is that species can be studied in their natural environment with fewer extrinsic factors acting on them, which may alter their natural response. In a laboratory setup, research is carried out in an artificial environment, with few of the sensory cues that would exist in the wild. Furthermore, most research is carried out in small tanks, which do not replicate the scale of the open ocean. Research in the field is the only realistic option to study community-level responses of marine species.

The aim of this study was to explore the impact of pile-driving noise on the behaviour of marine species in a natural setting, having already undertaken research in the laboratory on the physiology and behaviour of an invertebrate species. A field-based approach would help to better understand if there is a behavioural impact on marine species from pile-driving noise playback in the environment. The study looked at the effect of pile-driving playback on fish community composition *in situ* (which initially also investigated the effect on invertebrates). Video data were used to assess change in numbers of fish and species richness with the onset of pile-driving noise playback in comparison to ambient sound playback. Previous marine anthropogenic noise research has demonstrated behavioural changes in fish communities to motorboat passes (Mensinger *et al*., 2016). This study tested the hypothesis that there would be a significant decrease in community composition when exposed to pile-driving playback *in situ*, since fish were expected to avoid the impulsive pile-driving noise due to perception of the noise as a threat.

3.2 Materials and Methods

3.2.1 Experimental Design

A BRUV (baited remote underwater video) system was used in conjunction with noise playback to determine the community-level response of marine fauna to pile-driving noise *in situ*. On consecutive mornings from 1–5 August 2017, within 3 h of low water, the BRUV was dropped (n = 20) off a breakwater at Babbacombe Beach, UK (50°28'47" N, 03°30'30" W; Fig. 3.1). This was carried out at four different drop locations along the breakwater, with depth varying from 2–4 m.

Figure 3.1. Babbacombe beach and breakwater.

The BRUV consisted of a GoPro Hero5 Black video camera mounted on a metal base (58 x 38 cm) and a baited cage attached on a metal arm 50 cm in front of the video camera (Fig. 3.2). For each trial, half an Atlantic mackerel (*Scomber scombrus*; ~150 g) was placed in the bait cage. An Aqua-30 (DNH, 30 W, frequency range: 80–20,000 Hz) underwater loudspeaker was attached above the camera on the BRUV and was orientated towards the bait cage. The loudspeaker was connected to an 18 W amplifier (Kemo Electronic GmBH, frequency response; 40–20,000 Hz) with a potentiometer set to minimum resistance, all powered by a 12 V battery. A Clip Jam MP3 player (SanDisk, frequency range: 20–20,000 Hz) was used for noise playback of the different treatments.

Figure 3.2. The BRUV system.

Four WAV tracks were made for playback in this experiment using Audacity 2.1: two continuous ambient treatments (10 min of ambient sound followed by another 10 min of ambient sound) and two ambient to pile-driving treatments (10 min of ambient sound followed by 10 min of ambient sound with pile-driving noise mixed over the top). The original ambient sound was recorded from a RIB at Kimmeridge Bay, UK (50°36'22" N, 02°08'11" W) using an HTI-96-MIN hydrophone and an F8 Multitrack recorder (Zoom). The original pile-driving noise was recorded at Blyth, UK (55°08'46" N, 01°25'15" W) at approximately 120 m from a pile-driver; see Poulton *et al.* (2016) for further details.

For each trial, the camera was set to record, mackerel bait placed into the cage and then the BRUV with loudspeaker attached was lowered over the side of the breakwater wall at one of the four drop locations. After a settling period of 30 s on the seabed, the trial would begin with the chosen playback treatment started on the MP3 player and left to run for 20 min whilst recording video (Fig. 3.3). The continuous ambient treatments (n = 10) and the ambient to pile-driving treatments (n = 10) were randomly allocated for the different days and drop locations.

Figure 3.3. Image taken from BRUV *in situ*.

Videos were excluded for analysis if macroalgae or bad visibility obscured view of the bait cage and marine fauna (n = 2). Videos were analysed silently (so blind to the treatment) to avoid unintended bias in observations. The recordings were analysed for 5 min before and after the 10-min mark, where pile-driving would start to play in the ambient to pile-driving treatment or would continue in the continuous ambient treatment. Analysis was carried out 5 min in to allow for acclimation to the BRUV into the environment and a more similar bait condition. Videos were paused every 10 s in the two 5-min periods for each video and counts made of the number of different fish species and whether they were benthic or pelagic species. Fish were only counted if they could be reliably identified; this was kept consistent throughout the recording. Invertebrates were excluded from this analysis due to the difficulty of reliably landing the BRUV flat enough to the seabed. A list of observed fish species during the experiment is presented in Table 3.1.

Common Name	**Latin Name**	**Zone**
Ballan wrasse	*Labrus bergylta*	Pelagic
Pouting	*Trisopterus luscus*	Pelagic
Common blenny	*Lipophrys pholis*	Benthic
Corkwing wrasse	*Symphodus melops*	Pelagic
Goldsinny wrasse	*Ctenolabrus rupestris*	Pelagic
Atlantic Pollock	*Pollachius pollachius*	Pelagic
Rock cook	*Centrolabrus exoletus*	Pelagic
Rock goby	*Gobius paganellus*	Benthic
Sand smelt	*Atherina presbyter*	Pelagic
Tompot blenny	*Parablennius gattorugine*	Benthic

Table 3.1. Observed fish species.

From these data, percentage change in numbers of pelagic fish, numbers of benthic fish and species richness (number of different species) could be calculated for the different playback treatments; this controlled for baseline conditions for each drop site, accounting for natural variation between each of the drop sites when testing for a response to playback. Due to low power at species level, fish were grouped as benthic and pelagic fish for analysis (Table 3.1).

Statistical analyses were carried out using SPSS Statistics 24 (IBM). T-tests were used to compare data in the two treatments. Significance was assessed with an alpha-value of 0.05.

3.2.2 Acoustic Analysis

Waveforms were generated in Audacity 2.1 from original tracks for playback and *in situ* recordings made in Bude canal (Fig. 3.4).

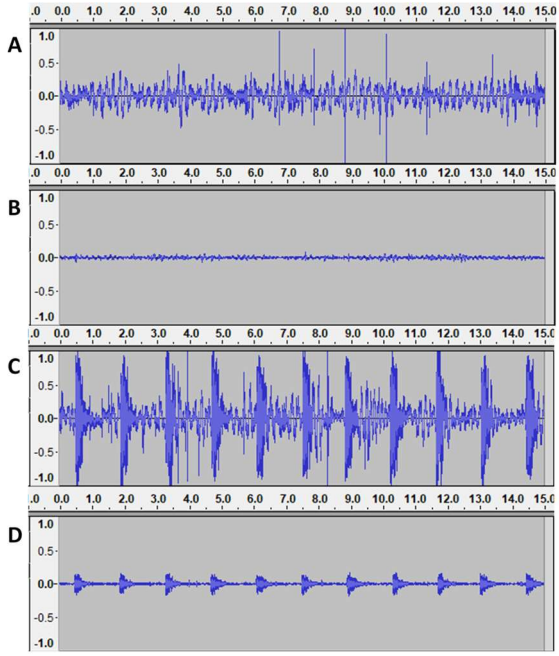

Figure 3.4. Examples of waveforms (15 s long) for A) original ambient treatment track, B) recording of ambient treatment playback *in situ*, C) original pile-driving treatment track, and D) recording of pile-driving treatment playback *in situ*.

Bude canal *in situ* sound recordings were analysed in MATLAB (V. 2013a) using paPAM (Nedelec *et al.*, 2016). Illustrative spectrograms (Fig. 3.5) were generated for 0-3,000 Hz, the most relevant frequency levels for crustaceans and fish (Lovell *et al.*, 2005; Popper and Hastings, 2009). These were calculated using a Hamming evaluation window, sample rate window length and 50% overlap over 10 s.

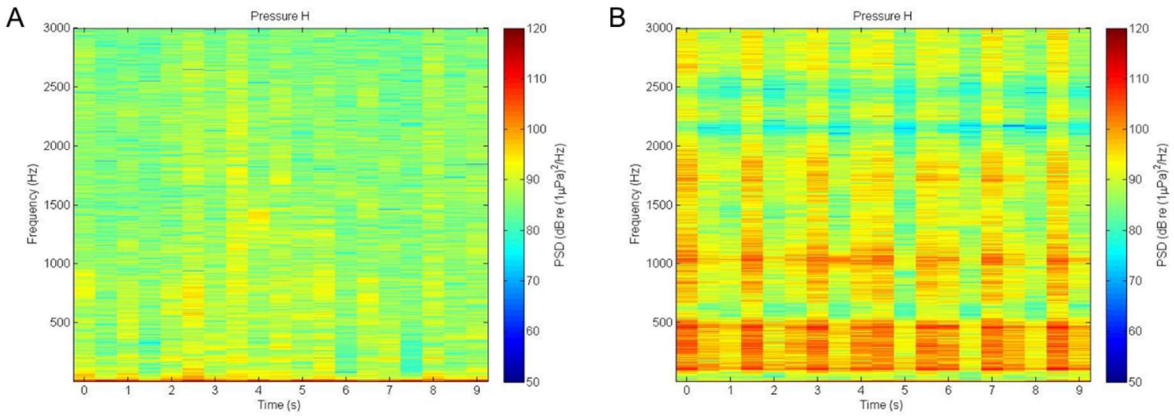

Figure 3.5. Illustrative spectrograms of A) ambient treatment playback *in situ*, and B) pile-driving treatment playback *in situ*.

3.3 Results

The mean percentage change in the number of benthic fish was not significantly different between the continuous ambient treatment and the ambient to pile-driving treatment (Independent t-test: $t_{16} = 0.868$, $p = 0.398$; Fig. 3.6).

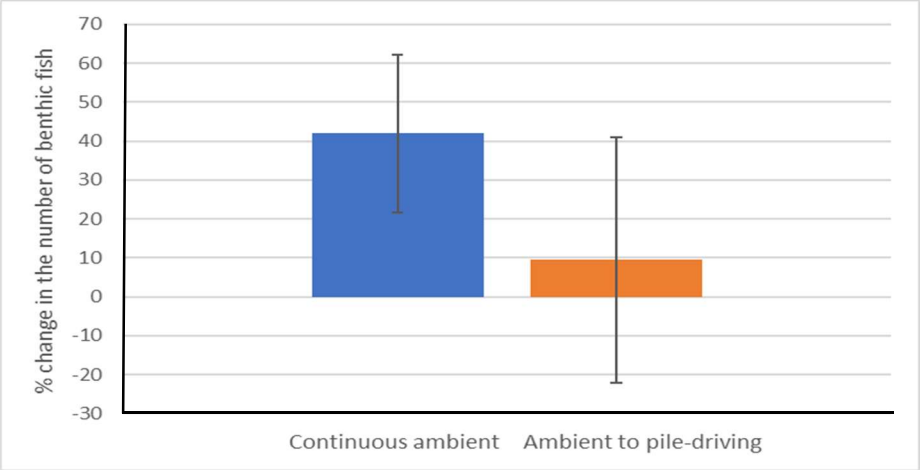

Figure 3.6. Mean ± SE percentage change in the number of benthic fish *in situ* after the 10-min mark when exposed to either the continuous ambient treatment (n = 9) or the ambient to pile-driving treatment (n = 9).

The mean percentage change in the number of pelagic fish was significantly greater in the continuous ambient treatment than in the ambient to pile-driving treatment (Independent t-test: $t_{16} = 2.531$, $p = 0.022$; Fig. 3.7).

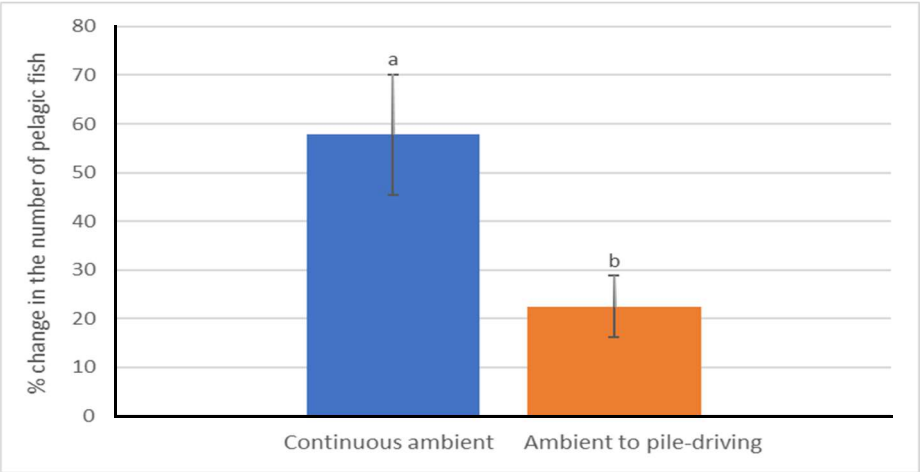

Figure 3.7. Mean ± SE percentage change in the number of pelagic fish *in situ* after the 10-min mark when exposed to either the continuous ambient treatment (n = 9) or the ambient to pile-driving treatment (n = 9). Letters denote significantly different treatments.

The mean percentage change in species richness was not significantly different between the continuous ambient treatment and the ambient to pile-driving treatment (Independent t-test: $t_{16} = 968$, $p = 0.347$; Fig. 3.8).

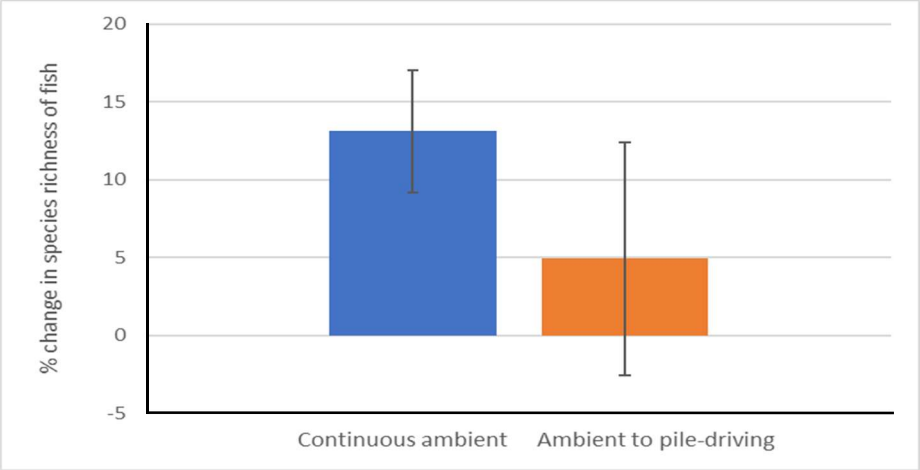

Figure 3.8. Mean ± SE percentage change in the species richness of fish *in situ* after the 10-min mark when exposed to either the continuous ambient treatment (n = 9) or the ambient to pile-driving treatment (n = 9).

3.4 Discussion

The aim of this experiment was to explore the impact of pile-driving playback on the community-level response of marine fish species in a natural setting. Playback of pile-driving noise had a significant effect on the avoidance behaviour of pelagic fish species surrounding the BRUV (baited remote underwater video) system. There was a significantly larger percentage increase in pelagic fish numbers in the continuous ambient treatment in comparison to the ambient to pile-driving treatment. There was also a greater increase in benthic fish numbers and species richness in the continuous ambient treatment compared to the pile-driving treatment, but the differences were not significantly different, potentially due to limited sample sizes. There have been a few studies previously into behavioural responses of fish to pile-driving noise in the ocean and the laboratory. Studies on fish have shown behavioural responses to pile-driving noise such as altered swimming speeds, increased freezing, less cohesion in groups and increased dispersal in a few cases (Mueller-Blenkle *et al.*, 2010; Hawkins *et al.,* 2014; Iafrate *et al.*, 2016; Herbert-Read *et al.*, 2017). There have been even fewer studies exploring the community-level effects of noise playback in the marine environment (Mensinger *et al*., 2016).

Despite a slight increase in pelagic fish on the ambient to pile-driving treatment, this was not as great as the increase in the continuous ambient treatment (Fig. 3.7), indicating a level of behavioural change to avoid the BRUV. This is similar to the findings from Mensinger *et al*. (2016) with BRUVs and motorboat noise, which explored abundance of fish, interactions with a bait jar and intraspecific interactions. During motorboat passes, behavioural changes were observed in fish, suggesting motorboat noise was the cause of this. However, continuous motorboat noise is different to impulsive pile-driving noise. Other studies have explored the impact of pile-driving noise on fish behaviour. For example, Atlantic cod, a pelagic species, showed an observable freezing behaviour in response to the onset of pile-driving noise in large net pens (Mueller-Blenkle *et al.*, 2010). European seabass in a laboratory experiment were found to be compromised in their shoaling behaviour, being less cohesive, less directionally ordered, and less correlated in speed and directional movements during pile-driving playback in comparison to ambient noise playback. Avoidance behaviour of pile-driving noise in pelagic fish has also been observed previously, with some fish changing depth

and others dispersing (Hawkins *et al.*, 2014; Iafrate *et al.*, 2016) These studies all demonstrate that fish have previously shown measurable behavioural effects in response to pile-driving noise, and so it is likely that pelagic fish in this experiment may have been deterred from the bait cage by the pile-driving noise playback.

The experimental noise playback *in situ* was shown to be different between the ambient and the pile-driving treatments, intense pile-driving peaks can be seen on the spectrogram for pile-driving playback, with intense peaks ranging from 0–3,000 Hz (Fig. 3.5). This falls within the range of frequencies that fish can detect (50–1500 Hz; Popper and Hastings, 2009). Pelagic fish such as the European pollock (*Pollachius pollachius*) in this study are able to detect sound using their inner ears and lateral line (Ladich, 2014). However, they also have a swim bladder which is primarily used as a buoyancy aid but is also utilised when detecting sound pressure as it is easily compressed by sound waves (Ladich, 2014). This would suggest that some fish are behaviourally avoiding the BRUV and the lure of the oily Atlantic mackerel bait due to being able to detect the pile-driving noise playback. It is possible pelagic fish may be responding to the pile-driving noise playback as an antipredator defence mechanism, perceiving the noise as a threat and choosing to avoid it. This avoidance behavioural response of pelagic fish species to pile-driving noise playback has significant implications for natural populations where real pile-driving would be much more intense over much greater distances. Behaviourally, pile-driving noise playback appears to have caused a degree of avoidance by pelagic fish within an area with food; if this was to carry on long-term this have potential have fitness costs by having reduced feeding success.

Since there was still a slight increase in pelagic fish in the ambient to pile-driving treatment is unexpected, this implies that not all fish avoided the pile-driving noise. One possibility is that even though they could detect the pile-driving noise they may not have been disturbed by it, possibly due to it not being as loud as real pile-driving would be close to the source. The lure of oily bait may have also overridden the avoidance behaviour in some fish as well, with fish condition and need for nutrition being a conflicting factor. The pelagic fish included a variety of species such as European pollock, Ballan wrasse (*Labrus bergylta*) and sand smelt (*Atherina presbyter*). Interspecific variation will occur with all fish having

different capabilities of detecting sound (Ladich, 2014); and will therefore detect the noise playback differently, and as a result are likely to respond differently. It is also possible that juvenile fish would be less capable of detecting sound due to a lack of hearing development and thus not respond to pile-driving playback. Previously, juvenile fish have been demonstrated to have reduced hearing capabilities compared to adult conspecifics (Wysocki and Ladich, 2001).

There was a greater percentage increase in the number of benthic fish surrounding the BRUV in the continuous ambient treatment in comparison to the ambient to pile-driving treatment, but, this was not a significant difference (Fig. 3.6). Low counts of benthic fish were observed during the trials which could result in a need for a greater sample size to pick up any statistical differences. However, this study indicates that avoidance behaviour in observed benthic fish such as the tompot blenny (*Parablennius gattorugine*) was not affected by pile-driving noise. The noise playback was, as stated above, within the general hearing range of fish (Popper and Hastings, 2009) but it is possible that it may not have been for these benthic fish. The most frequently seen benthic fish in this study included blennies and gobies such as the common blenny (*Lipophrys pholis*); it is possible that these fish have reduced hearing capabilities compared to other fish. This may be due to their anatomy – they have an inner ear – but often benthic fish do not have a swim bladder or it is a reduced size due to a benthic lifestyle (Helfman *et al.*, 2009). In a study on catfish, size of swim bladder was positively correlated with better hearing capabilities at higher frequencies (Lechner and Ladich, 2008). This reduced size or lack of swim bladder may have hindered detection of the pile-driving noise and may explain the lack of a significant difference in percentage change in fish between treatments.

There was a greater percentage increase in species richness on the BRUV recordings in the continuous ambient treatment in comparison to the ambient to pile-driving treatment, but it was not significantly different (Fig. 3.8). Similarly, to the benthic fish, there were low counts of different species observed during the trials which could result in a need for a greater sample size to detect statistical differences. It is also probable that numbers of each of the individual species would have been a better measure of avoidance behaviour due to interspecific variation in fish hearing.

In conclusion, the experiment has proven further that the BRUV system is a valuable ecological tool for field research on fish community-level effects, having been previously used in other anthropogenic noise studies (Mensinger *et al*., 2016; Roberts *et al*., 2016). Pile-driving noise playback did affect the avoidance behaviour of fish surrounding the BRUV, particularly for pelagic fish. This gives an indication as to the possible dispersal effect that real-world intense pile-driving activity could have on natural populations of pelagic fish, with potential fitness costs.

Not only could pile-driving noise cause problems for fish, it may impact humans directly. Fish are an extremely valuable ecological, commercial and social resource. It is estimated that fish provide more than 4.5 billion people with at least 15% of their intake of animal protein (Béné *et al.*, 2015), so conserving fish stocks for future generations is of the utmost importance. If there is a significant effect of pile-driving noise on fish communities, there are measures that can be undertaken to reduce the effect. This can be done temporally by avoiding breeding seasons of certain species or spatially by avoiding certain areas associated with vulnerable fish species. A reduction in noise in general would be ideal, with new technologies designed to reduce noise produced by pile-driving activity such as bubble curtains (Tsouvalas and Metrikine, 2016). Pile-driving industrial activity is only likely to increase in the marine environment in the future to tackle renewable energy targets. Better understanding of the effect that pile-driving is currently having on the marine environment will help to manage its impact in the future.

Chapter 4

Discussion – Limitations, Improvements and Future Directions

4.1 Limitations and Improvements – The Physiological and Behavioural Response of *Carcinus maenas* to Pile-driving Noise

The initial study into the physiological and behavioural response of *Carcinus maenas* to pile-driving noise drew interesting conclusions. It is important to note that the two experiments in this study have their limitations, primarily because both were conducted in the laboratory. While this approach provides tight control of experimental conditions, which is important for evidence-based research, it cannot replicate the potential response of animals in their natural environment. For example, in the laboratory, crabs will experience stress due to handling prior to exposure from pile-driving noise, resulting in conclusions about their responses being harder to assess. Therefore, ecological research is often carried out in the field to observe natural behaviours. The initial aim of the second study, using the BRUV system, was to follow up on the first study. This was to be carried out by observing behaviours of *C. maenas* and other marine invertebrates in addition to fish in a natural setting, in response to pile-driving noise. Unfortunately, there was difficulty in landing the BRUV system flat enough for marine invertebrates such as *C. maenas* to feed off the bait.

Small experimental tanks were used to carry out the laboratory-based study. In small tanks, sound fields are unrealistic of an open environment, as noise reverberates off tank walls causing sounds to become distorted (Okumura *et al.,* 2002). The experiments could have benefitted from being carried out in larger experimental tanks to give more realistic sound fields, by reducing reverberation off tank walls. However, the tanks would have to be very large to have any real effect on reverberations. Use of a real pile-driving rig in the laboratory is unfeasible, the best possible replication was to use playback through underwater loudspeakers of pile-driving noise.

A greater sample size would have benefitted both experiments and could be done if it was to be repeated. This would have helped to account for the effect of intra-population variation masking any differences between treatments. A small sample especially hindered one part of the behavioural experiment, preventing

valid statistical conclusions to be made on whether crabs were able to habituate or recover after pile-driving noise playback.

The life history of the wild-caught crabs is another consideration to assess. *C. maenas* used in this first study were collected from the Exe estuary in Exmouth. Previous research by Radford *et al.* (2016) demonstrated that exposure to pile-driving playback over 12 weeks led to a lessened physiological response in European seabass (*Dicentrarchus labrax*) to pile-driving noise and seismic noise, indicating a habituation to noise. This estuary is used frequently by small motorboats, so it is possible that the crabs may have been previously habituated to this noise type from the engines of these watercraft and thus had a reduced response to pile-driving noise due to habituation to all noise. Ideally, crabs would have been sourced from an area that is measurably quieter, although this would be difficult to assess without long-term recordings.

Furthermore, an accelerometer was not available when recording the playback conditions in the tanks, so acoustic conditions are reported only in the pressure domain. It would have been beneficial to have particle motion sound levels in the tanks due to it being the primary way that marine invertebrates detect sound (Nedelec *et al.*, 2016). However, particle motion measurements in small tanks do have their problems, especially due to the large accelerometer altering the particle motion field in the tank.

In the physiology study specifically, there are further improvements that could be made. A continuous oxygen reading rather than only start and end values would have also been beneficial to see if there was an initial or delayed onset of response, potentially followed by recovery. Whilst the crabs could not view each other, ideally, they should have been housed singly to avoid olfactory cues passing between crabs within each treatment. Additionally, the crustacean Hyperglycaemic Hormone (cHH) content of crab haemolymph could have been measured; this hormone stress response of crustaceans results in glucose being released in to the haemolymph (Lorenzon, 2005). It would be particularly useful to monitor rising levels of cHH as these can be seen much earlier than glucose increase in haemolymph (Webster, 1996). This could then have been compared to glucose concentrations in the haemolymph to draw conclusions on whether crabs were stressed by pile-driving playback.

4.2 Limitations and Improvements – The Marine Community-level Response to Pile-driving noise

This study was helpful to understand the response of wild fish to pile-driving noise, but it does also have some limitations. A greater sample size would have been more favourable to explore the differences in species richness and benthic fish. It would have also been advantageous to have used an accelerometer when recording noise playback as fish primarily detect the particle motion element of sound underwater (Nedelec *et al.*, 2016). An active pile-driving rig in the environment would have been ideal, but there is an obvious cost-related issue with this. In the future it may be possible to time research in the environment when actual industrial pile-driving is occurring offshore. The breakwater where this study was done was relatively quiet, with little boat traffic. It would have therefore been interesting to do a similar study at a harbour site which had more activity and see whether fish become habituated to the noise surrounding them, as previously demonstrated in another study into behavioural responses of fish to boat noise (Mensinger *et al*., 2016)

4.3 Future Directions

This research, even with the limitations described above, provides an insight into both the physiological and behavioural responses of marine species to pile-driving noise. Further research could be undertaken to further develop this field. Behavioural changes were most apparent in these studies, whether these would have long-term fitness costs if playback was continuous for a long period of time would be an interesting avenue of research to explore. As an extension of this study it would have been beneficial to expand on the second study and compare a noisy harbour against a quieter one as described previously (with either playback of pile-driving noise using a loudspeaker or an actual pile-driving rig). In the laboratory, it could be beneficial to look at a broader range of taxa, in particular from the wide range of marine invertebrates, which as a whole are underrepresented in pile-driving noise research. It may be particularly beneficial to study some of the commercial species, for instance *Cancer pagurus* which underpins a multi-million pound fishery in the UK. Better understanding of the impact of pile-driving noise on these species will help to inform decision-makers

on how to mitigate the potential impacts pile-driving noise is having on the fishing industry and on marine species in general.

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